**Fig S1 – supplementary for Method:**

pLL-SLAMF6-V1-3: vs Flag-VAR1

V1-A:

EMBOSS\_001 351 CCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTT 400

EMBOSS\_001 1 -------------------------------------------------- 0

EMBOSS\_001 401 TCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGC 450

EMBOSS\_001 1 -------------------------------------------------- 0

EMBOSS\_001 451 GTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGTCAG 500

EMBOSS\_001 1 -------------------------------------------------- 0

**(NheI/Xb)Kozak start**

EMBOSS\_001 501 ATCCGCTAGACC**ATG**TTGTGGCTGTTCCAATCGCTCCTGTTTGTCTTCTG 550

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EMBOSS\_001 1 ------------ATGTTGTGGCTGTTCCAATCGCTCCTGTTTGTCTTCTG 38

**Flag tag**

EMBOSS\_001 551 CTTTGGCCCAGGGAATGTAGTTTCAGACTACAAGGACGATGATGACAAGG 600

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EMBOSS\_001 39 CTTTGGCCCAGGGAATGTAGTTTCAGACTACAAGGACGATGATGACAAGG 88

**Linker**

EMBOSS\_001 601 GTTCAGGTCAAAGCAGCTTAACCCCATTGATGGTGAACGGGATTCTGGGG 650

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EMBOSS\_001 89 GTTCAGGTCAAAGCAGCTTAACCCCATTGATGGTGAACGGGATTCTGGGG 138

EMBOSS\_001 651 GAGTCAGTAACTCTTCCCCTGGAGTTTCCTGCAGGAGAGAAGGTCAACTT 700

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EMBOSS\_001 139 GAGTCAGTAACTCTTCCCCTGGAGTTTCCTGCAGGAGAGAAGGTCAACTT 188

EMBOSS\_001 701 CATCACTTGGCTTTTCAATGAAACATCTCTTGCCTTCATAGTACCCCATG 750

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EMBOSS\_001 189 CATCACTTGGCTTTTCAATGAAACATCTCTTGCCTTCATAGTACCCCATG 238

EMBOSS\_001 751 AAACCAAAAGTCCAGAAATCCACGTGACTAATCCGAAACAGGGAAAGCGA 800

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EMBOSS\_001 239 AAACCAAAAGTCCAGAAATCCACGTGACTAATCCGAAACAGGGAAAGCGA 288

EMBOSS\_001 801 CTGAACTTCACCCAGTCCTACTCCCTGCAACTCAGCAACCTGAAGATGGA 850

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EMBOSS\_001 289 CTGAACTTCACCCAGTCCTACTCCCTGCAACTCAGCAACCTGAAGATGGA 338

EMBOSS\_001 851 AGACACAGGCTCTTACAGAGCCCAGATATCCACAAAGACCTCTGCAAAGC 900

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EMBOSS\_001 339 AGACACAGGCTCTTACAGAGCCCAGATATCCACAAAGACCTCTGCAAAGC 388

EMBOSS\_001 901 TGTCCAGTTACACTCTGAGGATATTAAGACAACTGAGGAACATACAAGTT 950

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EMBOSS\_001 389 TGTCCAGTTACACTCTGAGGATATTAAGACAACTGAGGAACATACAAGTT 438

EMBOSS\_001 951 ACCAATCACAGTCAGCTATTTCAGAATATGACCTGTGAGCTCCATCTGAC 1000

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EMBOSS\_001 439 ACCAATCACAGTCAGCTATTTCAGAATATGACCTGTGAGCTCCATCTGAC 488

EMBOSS\_001 1001 TTGCTCTGTGGAGGATGCAGATGACAATGTCTCA-TCAGAT--GAGTCNT 1047

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EMBOSS\_001 489 TTGCTCTGTGGAGGATGCAGATGACAATGTCTCATTCAGATGGGAGGCCT 538

pLL3:

EMBOSS\_001 4 TTACAGAGCCCCAGATATCCACAAAGCACATCTGCAAAAGCTGTCCAGTT 53

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EMBOSS\_001 351 TTACAGAG-CCCAGATATCCACAAAG-ACCTCTGC-AAAGCTGTCCAGTT 397

EMBOSS\_001 54 ACA-TCTGAAGGATATTAAGACAACTGAGGAACATACAAAGTTACCAATC 102

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EMBOSS\_001 398 ACACTCTG-AGGATATTAAGACAACTGAGGAACATAC-AAGTTACCAATC 445

EMBOSS\_001 103 ACAGTCAGCTATTTCAGAATATGACCTGTGAGCTCCATCTGACTTGCTCT 152

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EMBOSS\_001 446 ACAGTCAGCTATTTCAGAATATGACCTGTGAGCTCCATCTGACTTGCTCT 495

EMBOSS\_001 153 GTGGAGGATGCAGATGACAATGTCTCATTCAGATGGGAGGCCTTGGGAAA 202

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EMBOSS\_001 496 GTGGAGGATGCAGATGACAATGTCTCATTCAGATGGGAGGCCTTGGGAAA 545

EMBOSS\_001 203 CACACTTTCAAGTCAGCCAAACCTCACTGTCTCCTGGGACCCCAGGATTT 252

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EMBOSS\_001 546 CACACTTTCAAGTCAGCCAAACCTCACTGTCTCCTGGGACCCCAGGATTT 595

EMBOSS\_001 253 CCAGTGAACAGGACTACACCTGCATAGCAGAGAATGCTGTCAGTAATTTA 302

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EMBOSS\_001 596 CCAGTGAACAGGACTACACCTGCATAGCAGAGAATGCTGTCAGTAATTTA 645

EMBOSS\_001 303 TCCTTCTCTGTCTCTGCCCAGAAGCTTTGCGAAGATGTTAAAATTCAATA 352

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EMBOSS\_001 646 TCCTTCTCTGTCTCTGCCCAGAAGCTTTGCGAAGATGTTAAAATTCAATA 695

EMBOSS\_001 353 TACAGATACCAAAATGATTCTGTTTATGGTTTCTGGGATATGCATAGTCT 402

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EMBOSS\_001 696 TACAGATACCAAAATGATTCTGTTTATGGTTTCTGGGATATGCATAGTCT 745

EMBOSS\_001 403 TCGGTTTCATCATACTGCTGTTACTTGTTTTGAGGAAAAGAAGAGATTCC 452

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EMBOSS\_001 746 TCGGTTTCATCATACTGCTGTTACTTGTTTTGAGGAAAAGAAGAGATTCC 795

EMBOSS\_001 453 CTATCTTTGTCTACTCAGCGAACACAGGGCCCCGCAGAGTCCGCAAGGAA 502

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EMBOSS\_001 796 CTATCTTTGTCTACTCAGCGAACACAGGGCCCCGCAGAGTCCGCAAGGAA 845

EMBOSS\_001 503 CCTAGAGTATGTTTCAGTGTCTCCAACGAACAACACTGTGTATGCTTCAG 552

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EMBOSS\_001 846 CCTAGAGTATGTTTCAGTGTCTCCAACGAACAACACTGTGTATGCTTCAG 895

EMBOSS\_001 553 TCACTCATTCAAACAGGGAAACAGAAATCTGGACACCTAGAGAAAATGAT 602

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EMBOSS\_001 896 TCACTCATTCAAACAGGGAAACAGAAATCTGGACACCTAGAGAAAATGAT 945

EMBOSS\_001 603 ACTATCACAATTTACTCCACAATTAATCATTCCAAAGAGAGTAAACCCAC 652

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EMBOSS\_001 946 ACTATCACAATTTACTCCACAATTAATCATTCCAAAGAGAGTAAACCCAC 995

**Stop EcoRI**

EMBOSS\_001 653 TTTTTCCAGGGCAACTGCCCTTGACAATGTCGTGTAAGAATTCGTCGAGG 702

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EMBOSS\_001 996 TTTTTCCAGGGCAACTGCCCTTGACAATGTCGTGTAA------------- 1032

EMBOSS\_001 703 GACCTAATAACTTCGTATAGCATACATTATACGAAGTTATACAGTAAGGT 752

pLL-SLAMF6-V3-1: vs Flag-VAR3

VAR3-A:

EMBOSS\_001 801 TCAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAG 850

EMBOSS\_001 1 -------------------------------------------------- 0

**(NheI/Xb)Kozak start**

EMBOSS\_001 851 GTCTATATAAGCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGACC**AT** 900

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EMBOSS\_001 1 ------------------------------------------------AT 2

EMBOSS\_001 901 **G**TTGTGGCTGTTCCAATCGCTCCTGTTTGTCTTCTGCTTTGGCCCAGTAC 950

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EMBOSS\_001 3 GTTGTGGCTGTTCCAATCGCTCCTGTTTGTCTTCTGCTTTGGCCCAGTAC 52

**Flag tag Linker**

EMBOSS\_001 951 CCCATGAAACCGACTACAAGGACGATGATGACAAGGGTTCAGGTAAAAGT 1000

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EMBOSS\_001 53 CCCATGAAACCGACTACAAGGACGATGATGACAAGGGTTCAGGTAAAAGT 102

EMBOSS\_001 1001 CCAGAAATCCACGTGACTAATCCGAAACAGGGAAAGCGACTGAACTTCAC 1050

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EMBOSS\_001 103 CCAGAAATCCACGTGACTAATCCGAAACAGGGAAAGCGACTGAACTTCAC 152

EMBOSS\_001 1051 CCAGTCCTACTCCCTGCAACTCAGCAACCTGAAGATGGAAGACACAGGCT 1100

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EMBOSS\_001 153 CCAGTCCTACTCCCTGCAACTCAGCAACCTGAAGATGGAAGACACAGGCT 202

EMBOSS\_001 1101 CTTACAGAGCCCAGATATCCACAAAGACCTCTGCAAAGCTGTCCAGTTAC 1150

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EMBOSS\_001 203 CTTACAGAGCCCAGATATCCACAAAGACCTCTGCAAAGCTGTCCAGTTAC 252

EMBOSS\_001 1151 ACTCTGAGGATATTAAGACAACTGAGGAACATACAAGTTACCAATCACAG 1200

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EMBOSS\_001 253 ACTCTGAGGATATTAAGACAACTGAGGAACATACAAGTTACCAATCACAG 302

EMBOSS\_001 1201 TCAGCTATTTCAGAATATGACCTGTGAGCTCCATCTGACTTGCTCTGTGG 1250

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EMBOSS\_001 303 TCAGCTATTTCAGAATATGACCTGTGAGCTCCATCTGACTTGCTCTGTGG 352

EMBOSS\_001 1251 AGGATGCAGATGACAATGTCTCA-TCAGATGAG------TCNGA------ 1287

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EMBOSS\_001 353 AGGATGCAGATGACAATGTCTCATTCAGATGGGAGGCCTTGGGAAACACA 402

pLL3:

EMBOSS\_001 301 CAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGACCATGTTGTGGCTGT 350

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EMBOSS\_001 1 -------------------------------------ATGTTGTGGCTGT 13

EMBOSS\_001 351 TCCAATCGCTCCTGTTTGTCTTCTGCTTTGGCCCAGTACCCCATGAAACC 400

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EMBOSS\_001 14 TCCAATCGCTCCTGTTTGTCTTCTGCTTTGGCCCAGTACCCCATGAAACC 63

EMBOSS\_001 401 GACTACAAGGACGATGATGACAAGGGTTCAGGTAAAAGTCCAGAAATCCA 450

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EMBOSS\_001 64 GACTACAAGGACGATGATGACAAGGGTTCAGGTAAAAGTCCAGAAATCCA 113

EMBOSS\_001 451 CGTGACTAACCCGAAACAGGGAAAGCGACTGAACTTCACCCAGTCCTACT 500

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EMBOSS\_001 114 CGTGACTAATCCGAAACAGGGAAAGCGACTGAACTTCACCCAGTCCTACT 163

EMBOSS\_001 501 CCCTGCAACTCAGCAACCTGAAGATGGAAGACACAGGCTCTTACAGAGCC 550

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EMBOSS\_001 164 CCCTGCAACTCAGCAACCTGAAGATGGAAGACACAGGCTCTTACAGAGCC 213

EMBOSS\_001 551 CAGATATCCACAAAGACCTCTGCAAAGCTGTCCAGTTACACTCTGAGGAT 600

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EMBOSS\_001 214 CAGATATCCACAAAGACCTCTGCAAAGCTGTCCAGTTACACTCTGAGGAT 263

EMBOSS\_001 601 ATTAAGACAACTGAGGAACATACAAGTTACCAATCACAGTCAGCTATTTC 650

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EMBOSS\_001 264 ATTAAGACAACTGAGGAACATACAAGTTACCAATCACAGTCAGCTATTTC 313

EMBOSS\_001 651 AGAATATGACCTGTGAGCTCCATCTGACTTGCTCTGTGGAGGATGCAGAT 700

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EMBOSS\_001 314 AGAATATGACCTGTGAGCTCCATCTGACTTGCTCTGTGGAGGATGCAGAT 363

EMBOSS\_001 701 GACAATGTCTCATTCAGATGGGAGGCCTTGGGAAACACACTTTCAAGTCA 750

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EMBOSS\_001 364 GACAATGTCTCATTCAGATGGGAGGCCTTGGGAAACACACTTTCAAGTCA 413

EMBOSS\_001 751 GCCAAACCTCACTGTCTCCTGGGACCCCAGGATTTCCAGTGAACAGGACT 800

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EMBOSS\_001 414 GCCAAACCTCACTGTCTCCTGGGACCCCAGGATTTCCAGTGAACAGGACT 463

EMBOSS\_001 801 ACACCTGCATAGCAGAGAATGCTGTCAGTAATTTATCCTTCTCTGTCTCT 850

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EMBOSS\_001 464 ACACCTGCATAGCAGAGAATGCTGTCAGTAATTTATCCTTCTCTGTCTCT 513

EMBOSS\_001 851 GCCCAGAAGCTTTGCGAAGATGTTAAAATTCAATATACAGATACCAAAAT 900

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EMBOSS\_001 514 GCCCAGAAGCTTTGCGAAGATGTTAAAATTCAATATACAGATACCAAAAT 563

EMBOSS\_001 901 GATTCTGTTTATGGTTTCTGGGATATGCATAGTCTTCGGTTTCATCATAC 950

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EMBOSS\_001 564 GATTCTGTTTATGGTTTCTGGGATATGCATAGTCTTCGGTTTCATCATAC 613

EMBOSS\_001 951 TGCTGTTACTTGTTTTGAGGAAAAGAAGAGATTCCCTATCTTTGTCTACT 1000

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EMBOSS\_001 614 TGCTGTTACTTGTTTTGAGGAAAAGAAGAGATTCCCTATCTTTGTCTACT 663

EMBOSS\_001 1001 CAGCGAACACAGGGCCCCGAGTCCGCAAGGAACCTAGAGTATGTTTCAGT 1050

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EMBOSS\_001 664 CAGCGAACACAGGGCCCCGAGTCCGCAAGGAACCTAGAGTATGTTTCAGT 713

EMBOSS\_001 1051 GTCTCCAACGAACAACACTGTGTATGCTTCAGTCACTCATTCAAACAGGG 1100

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EMBOSS\_001 714 GTCTCCAACGAACAACACTGTGTATGCTTCAGTCACTCATTCAAACAGGG 763

EMBOSS\_001 1101 AAACAGAAATCTGGACACCTAGAGAAAATGATACTATCACAATTTACTCC 1150

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EMBOSS\_001 764 AAACAGAAATCTGGACACCTAGAGAAAATGATACTATCACAATTTACTCC 813

EMBOSS\_001 1151 ACAATTAATCATTCCAAAGAGAGTAAACCCACTTTTTCCAGGGCAACTGC 1200

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EMBOSS\_001 814 ACAATTAATCATTCCAAAGAGAGTAAACCCACTTTTTCCAGGGCAACTGC 863

**Stop EcoRI**

EMBOSS\_001 1201 CCTTGACAATGTCGTGTAAGAATTCGTCGAGGGACCTAATAACTTCGTAT 1250

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EMBOSS\_001 864 CCTTGACAATGTCGTGTAA------------------------------- 882

EMBOSS\_001 1251 AGCATACATTATACGAAGTTATACAGTAACGTCCTCCGC 1289

pLL-SLAMF6-V4-4: vs Flag-VAR4

pLL3:

EMBOSS\_001 151 ACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTC 200

EMBOSS\_001 1 -------------------------------------------------- 0

**(NheI/Xb)Kozak start**

EMBOSS\_001 201 TATATAAGCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGACC**ATG**TT 250

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EMBOSS\_001 1 ---------------------------------------------ATGTT 5

EMBOSS\_001 251 GTGGCTGTTCCAATCGCTCCTGTTTGTCTTCTGCTTTGGCCCAGGACAAC 300

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EMBOSS\_001 6 GTGGCTGTTCCAATCGCTCCTGTTTGTCTTCTGCTTTGGCCCAGGACAAC 55

**Flag tag Linker**

EMBOSS\_001 301 TGGACTACAAGGACGATGATGACAAGGGTTCAGGTAGGAACATACAAGTT 350

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EMBOSS\_001 56 TGGACTACAAGGACGATGATGACAAGGGTTCAGGTAGGAACATACAAGTT 105

EMBOSS\_001 351 ACCAATCACAGTCAGCTATTTCAGAATATGACCTGTGAGCTCCATCTGAC 400

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EMBOSS\_001 106 ACCAATCACAGTCAGCTATTTCAGAATATGACCTGTGAGCTCCATCTGAC 155

EMBOSS\_001 401 TTGCTCTGTGGAGGATGCAGATGACAATGTCTCATTCAGATGGGAGGCCT 450

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EMBOSS\_001 156 TTGCTCTGTGGAGGATGCAGATGACAATGTCTCATTCAGATGGGAGGCCT 205

EMBOSS\_001 451 TGGGAAACACACTTTCAAGTCAGCCAAACCTCACTGTCTCCTGGGACCCC 500

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EMBOSS\_001 206 TGGGAAACACACTTTCAAGTCAGCCAAACCTCACTGTCTCCTGGGACCCC 255

EMBOSS\_001 501 AGGATTTCCAGTGAACAGGACTACACCTGCATAGCAGAGAATGCTGTCAG 550

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EMBOSS\_001 256 AGGATTTCCAGTGAACAGGACTACACCTGCATAGCAGAGAATGCTGTCAG 305

EMBOSS\_001 551 TAATTTATCCTTCTCTGTCTCTGCCCAGAAGCTTTGCGAAGATGTTAAAA 600

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EMBOSS\_001 306 TAATTTATCCTTCTCTGTCTCTGCCCAGAAGCTTTGCGAAGATGTTAAAA 355

EMBOSS\_001 601 TTCAATATACAGATACCAAAATGATTCTGTTTATGGTTTCTGGGATATGC 650

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EMBOSS\_001 356 TTCAATATACAGATACCAAAATGATTCTGTTTATGGTTTCTGGGATATGC 405

EMBOSS\_001 651 ATAGTCTTCGGTTTCATCATACTGCTGTTACTTGTTTTGAGGAAAAGAAG 700

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EMBOSS\_001 406 ATAGTCTTCGGTTTCATCATACTGCTGTTACTTGTTTTGAGGAAAAGAAG 455

EMBOSS\_001 701 AGATTCCCTATCTTTGTCTACTCAGCGAACACAGGGCCCCGCAGAGTCCG 750

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EMBOSS\_001 456 AGATTCCCTATCTTTGTCTACTCAGCGAACACAGGGCCCCGCAGAGTCCG 505

EMBOSS\_001 751 CAAGGAACCTAGAGTATGTTTCAGTGTCTCCAACGAACAACACTGTGTAT 800

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EMBOSS\_001 506 CAAGGAACCTAGAGTATGTTTCAGTGTCTCCAACGAACAACACTGTGTAT 555

EMBOSS\_001 801 GCTTCAGTCACTCATTCAAACAGGGAAACAGAAATCTGGACACCTAGAGA 850

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EMBOSS\_001 556 GCTTCAGTCACTCATTCAAACAGGGAAACAGAAATCTGGACACCTAGAGA 605

EMBOSS\_001 851 AAATGATACTATCACAATTTACTCCACAATTAATCATTCCAAAGAGAGTA 900

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EMBOSS\_001 606 AAATGATACTATCACAATTTACTCCACAATTAATCATTCCAAAGAGAGTA 655

**Stop EcoRI**

EMBOSS\_001 901 AACCCACTTTTTCCAGGGCAACTGCCCTTGACAATGTCGTGTAAGAATTC 950

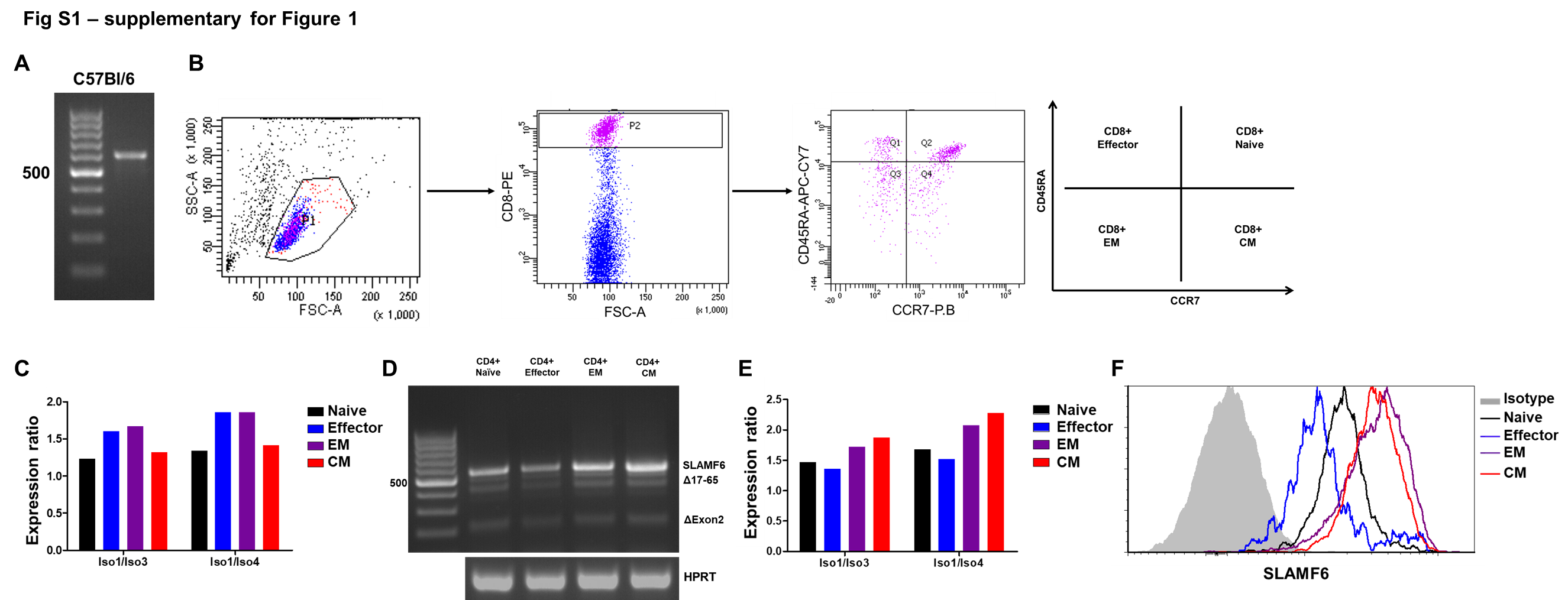
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EMBOSS\_001 656 AACCCACTTTTTCCAGGGCAACTGCCCTTGACAATGTCGTGTAA------ 699

EMBOSS\_001 951 GTCGAGGGACCTAATAACTTCGTATAGCATACATTATACGAAGTATACAG 1000

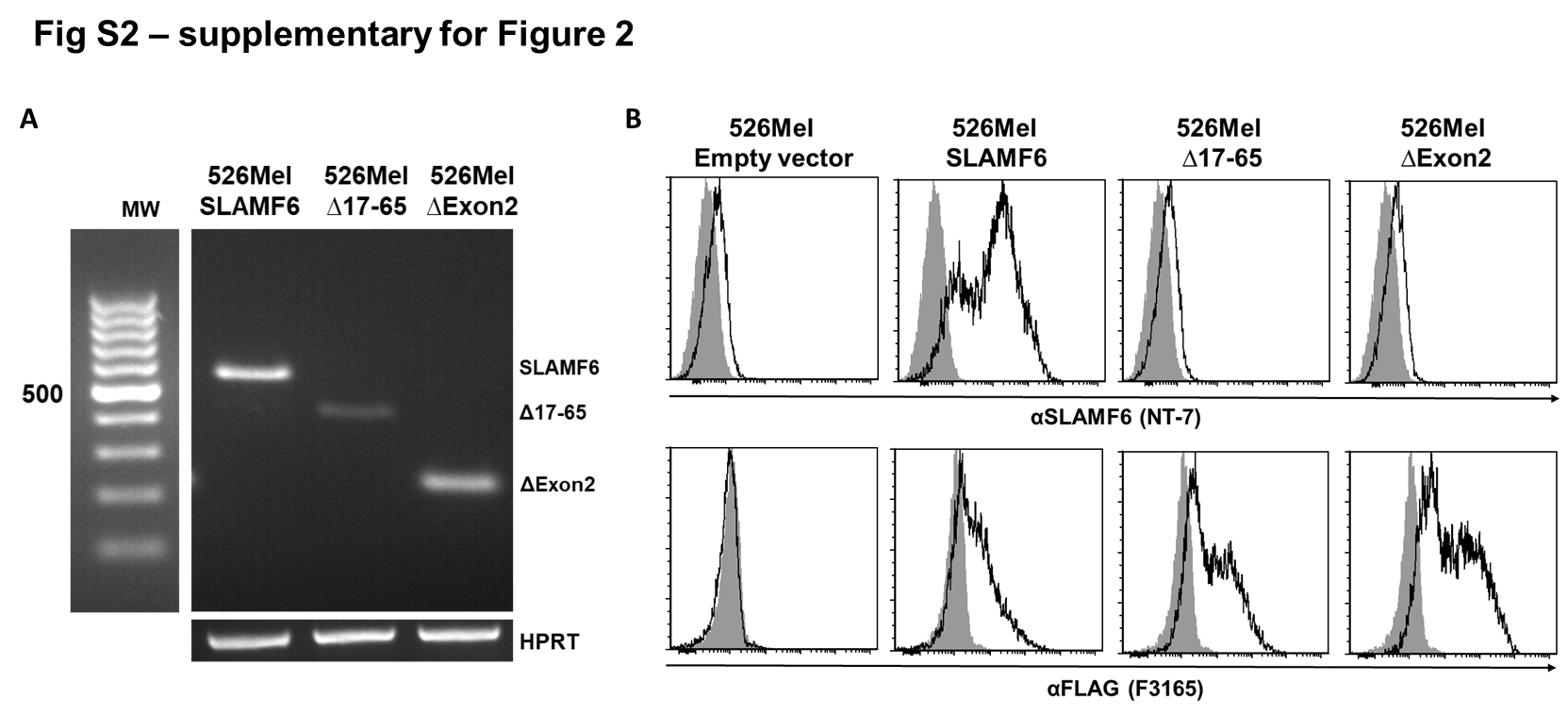
**Fig S1 – supplementary for Method:**

Sequencing files of the 3 plasmids used (3 SLAMF6 variants in PLL3.7 plasmid)



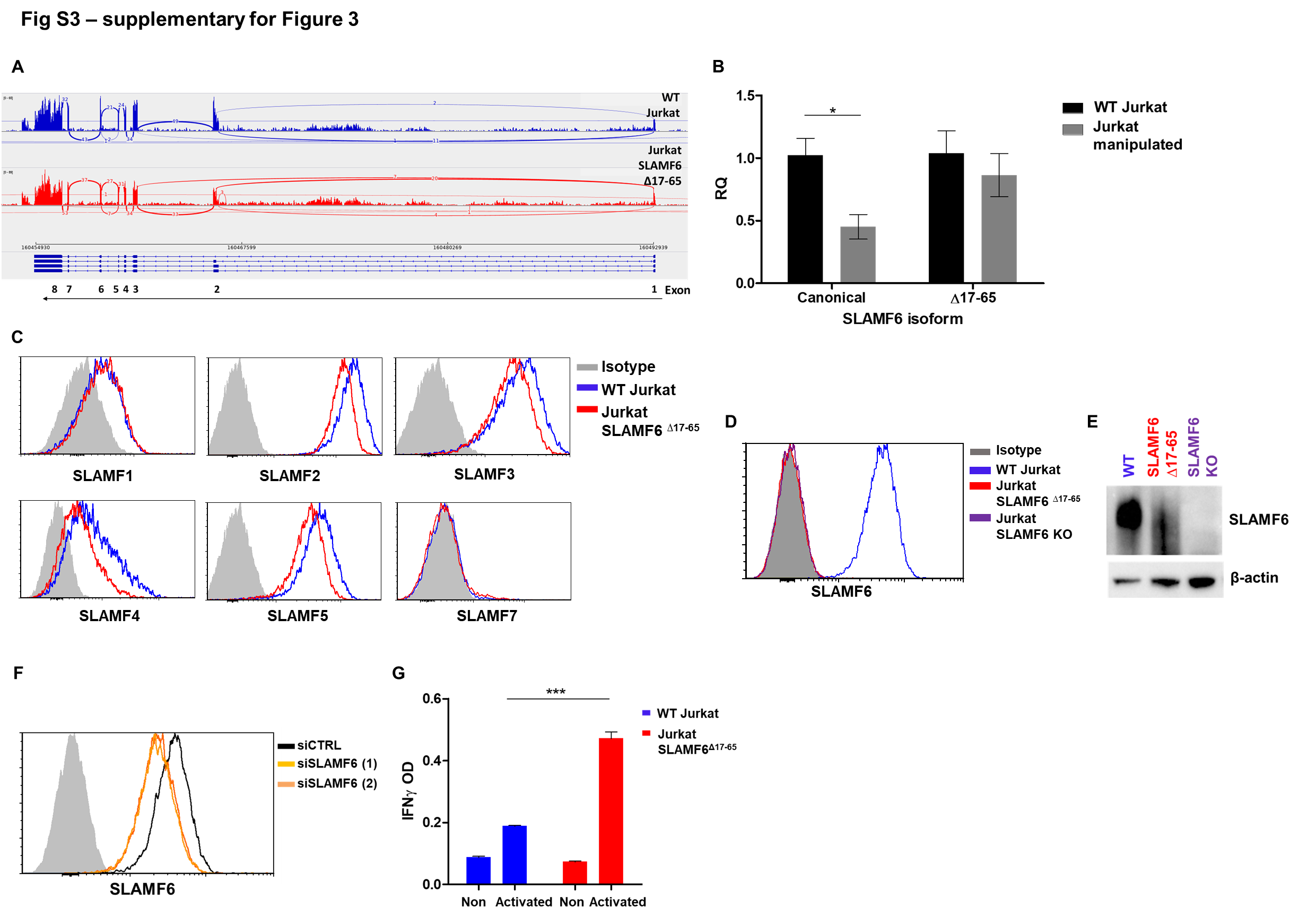
**Fig S2 – supplementary for Figure 1:**

**A**, RNA expression of *LY108*, the murine analog of *SLAMF6*, in C57Bl/6 splenocytes. **B**, Gating strategy for cell sorting separating CD8 T cells to subsets according to CD45RA/CCR7 expression. **C**, Ratio of isoforms as quantitated from Figure 1E. **D-F**, SLAMF6 isoform in naïve, effector, EM, and CM CD4 T cell subsets. **D**, RNA expression. **E**, Ratio of isoforms as quantitated from D. **F**, Flow cytometry of SLAMF6. EM, effector memory. CM, central memory.

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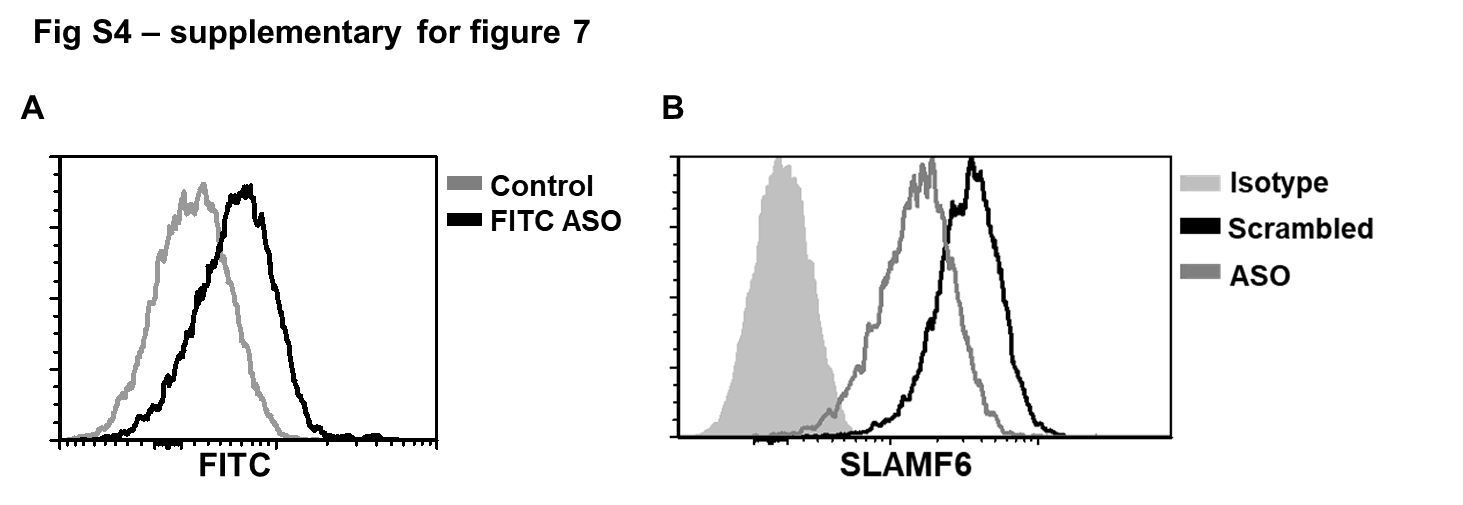
**Fig S3 – supplementary for Figure 2:**

Validation of SLAMF6 protein expression in modified melanoma cells. **A**, RNA expression of *SLAMF6* isoforms on transfected 526*mel* melanoma cells. **B**, Flow cytometry to determine SLAMF6 (MoAb NT-7) or FLAG (MoAb F3165) expression on the transfected 526*mel* cells.

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**Fig S4 – supplementary for Figure 3:**

**A**, Sashimi plot showing SLAMF6 splice isoforms in WT Jurkat cells and Jurkat-SLAMF6∆17-65 cells 36 h post-activation. **B**, Quantitative RT-PCR for *SLAMF6-canonical* and *SLAMF6*∆17-65 in relevant Jurkat cells. Data were normalized to *HPRT* expression and WT Jurkat expression. **C**, Expression of SLAM family members in WT Jurkat and Jurkat-SLAMF6∆17-65 cells. **D**,SLAMF6 canonical isoform expression in three Jurkat cell lines: WT, SLAMF6∆17-65 , and SLAMF6 KO. **E**, Immunoblot of SLAMF6 using anti-SLAMF6 (AF1908) targeting SLAMF6 C-domain. **F**, SLAMF6 canonical isoform silencing in WT Jurkat cells electroporated with siSLAMF6. **G**, ELISA for IFNγ in WT Jurkat and Jurkat-SLAMF6∆17-65 cells activated for 48 h. OD, optical density. One-way ANOVA. \*\*\*, P < 0.001.



**Fig S5 – supplementary for Figure 7:**

**A**, FITC levels measured by flow cytometry 24 h post electroporation of WT Jurkat cells with ASO-FITC or control ASO. **B**, SLAMF6 expression measured using flow cytometry 24 h post-electroporation of WT Jurkat cells with ASO or control ASO.