

## SUPPLEMENTARY INFORMATION

### Laminin $\beta$ 3 cDNA constructs and subcloning

Domain VI of the human laminin  $\beta$ 3 chain (*HuLAMB3*) spans amino acid residues 1-232 and domain III/V residues 233-561 (1). The first deletion construct ( $\Delta$ VI) begins at residue 229 thereby containing four amino acids of domain VI, one of which was changed during creation of an *Nhe* I site for cloning purposes (residue 230, a glycine, was exchanged for an alanine). The sequence encoding domain III/V begins with cysteine at aa 233 and the construct ends with the C-terminal stop codon of *HuLAMB3*, (aa 1153), encompassing domains I and II. Three pairs of PCR primers (**supplementary Table 1**): “BM40 forward” and “BM40 reverse”, “*Nhe* I VI forward” and “*Acc* I reverse”, and “*Acc* I forward” and “LAMB3 Stop reverse” were used to generate three cDNA fragments, which were then ligated together in Zero Blunt® TOPO® via *Hind* III, *Nhe* I, *Acc* I and *Not* I restriction digestions (**Supplementary Fig. 1**). An *Acc* I site, which cuts between aa 581 and aa 582, already existed in the *HuLAMB3* sequence and was exploited during subcloning. The PCR was performed using Pfu Ultra Hot Start polymerase as per the manufacturer’s instructions (Stratagene, La Jolla, CA) and a correct clone was verified by sequencing the full length of the assembled insert.

A second deletion mutant,  $\Delta$ VI-III, begins at residue 560, which for cloning purposes was exchanged from a valine to a leucine to create an *Nhe* I site. The sequence encoding domain II begins at aa 562. The fragments for subcloning were generated by PCR as described above, except that the primer “*Nhe* I VI-III Forward” was used in place of “*Nhe* I VI Forward”.

The deletion constructs, fused to a BM40 signal sequence, were sub-cloned into the *Eco*R I sites of pENTR™ 1A (Invitrogen, Carlsbad, CA) and then transferred into the

retroviral vector pLZRS-GATEWAY via a GATEWAY<sup>®</sup> recombination reaction (Invitrogen).

**Supplementary Table 1: PCR primers used to synthesize laminin  $\beta$ 3 cDNA constructs**

| Primer  | Sequence                                      |
|---|---|
| BM40 ( <i>Hind</i> III) <sup>1</sup> forward    | 5'-GCCAGCTGATCA <u>AAGCTT</u> CT-3'           |
| BM40 reverse                                    | 5'-CTTGCAGGGGTCTTTGTAGT-3'                    |
| <i>Nhe</i> I VI forward                         | 5'-CAT <u>GCTAGC</u> AGGGAGCTGCTTCTGTCAC-3'   |
| <i>Nhe</i> I VI-III forward                     | 5'-CAT <u>GCTAGC</u> CCTGCCACCCTTGCTTCCAG-3'  |
| <i>Acc</i> I reverse                            | 5'-TGGCATTGCGGAGTCTACCAAAGC-3'                |
| <i>Acc</i> I forward                            | 5'-CGCTTTG <u>GTAGACT</u> CCGCAATGCCAC-3'     |
| LAMB3 stop <sup>2</sup> ( <i>Not</i> I) reverse | 5'-TAGCGGCCGCT <b>TCA</b> CTTGCAGGTGGCATAG-3' |

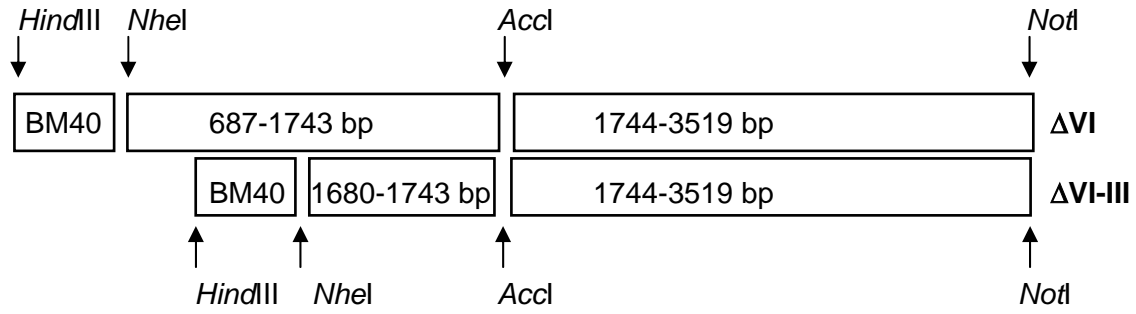
<sup>1</sup>Restriction sites are underlined

<sup>2</sup>Stop codon is shown in bold.

### Supplementary Figure Legend

**Figure 1.** Laminin  $\beta 3$  cDNAs used in generating  $\Delta VI$  and  $\Delta VI$ -III constructs. Numbers in boxes indicate position in base pairs (bp) in the *LAMB3* gene. Arrows indicate restriction enzyme sites used during subcloning.

## Supplementary Figure 1



## Reference

1. Gerecke DR, Wagman DW, Champlaud MF, Burgeson RE. The complete primary structure for a novel laminin chain, the laminin B1k chain. J Biol Chem. 1994;269:11073-80.