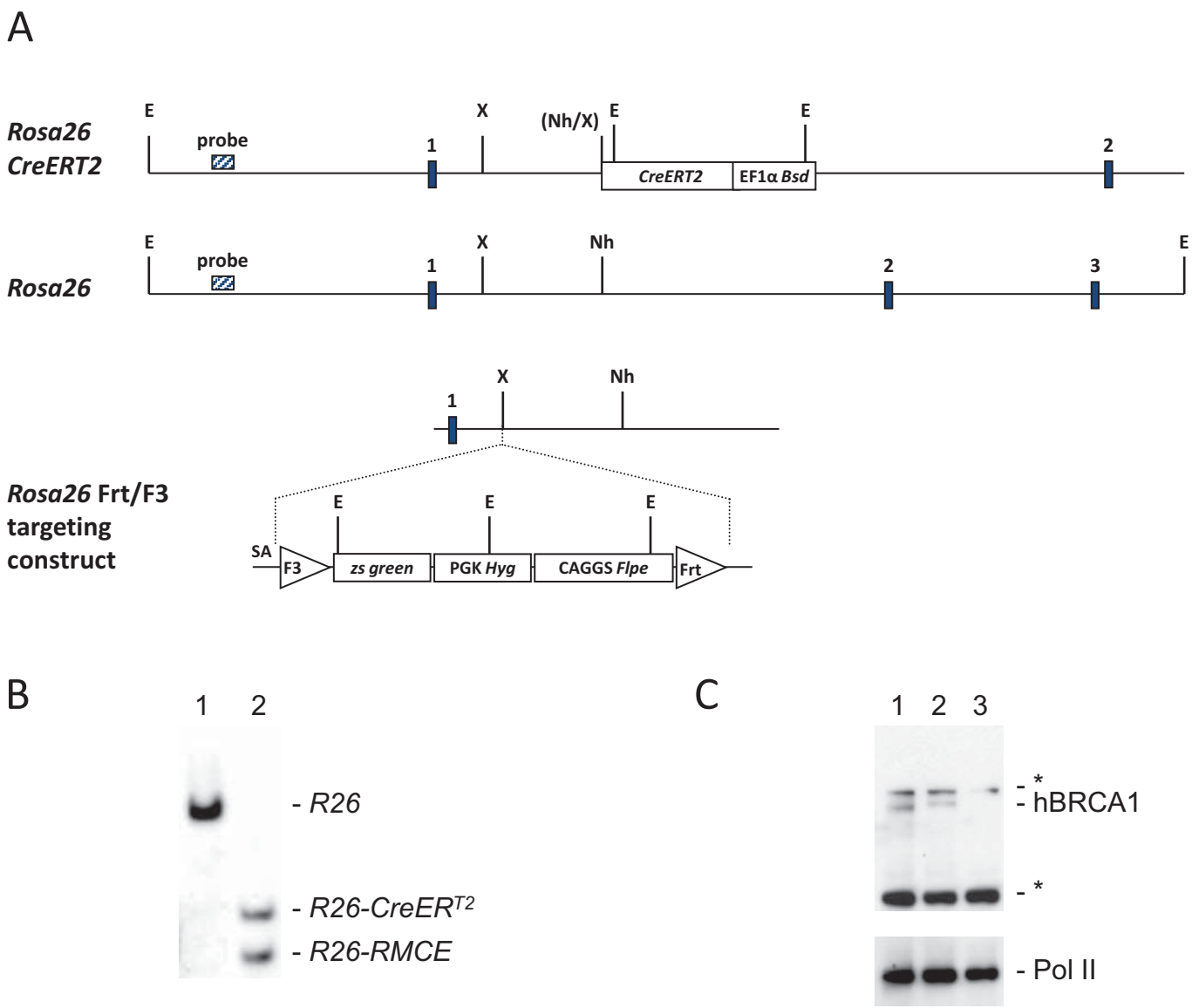
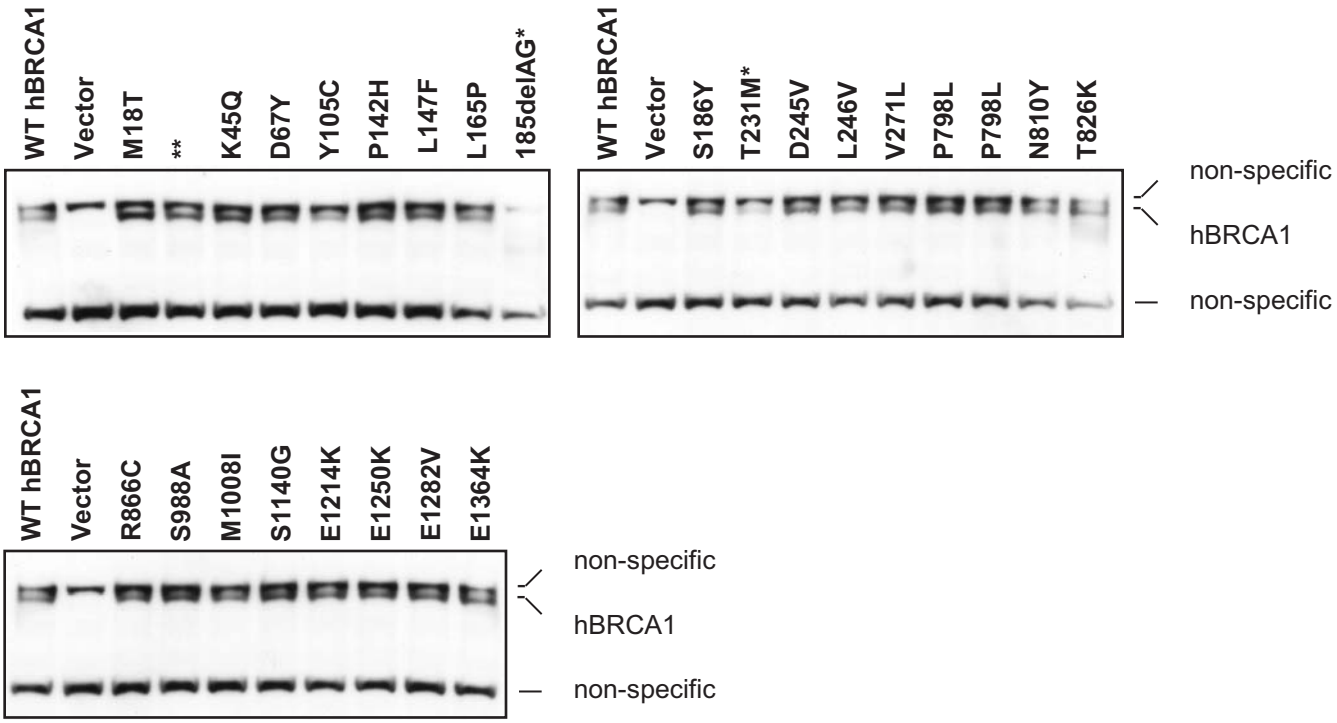
Generation of R26$^{CreERT2/RMCE}$;Brca1$^{SCo/\Delta}$ ES cells and analysis of Rosa26 targeted human BRCA1 cDNA expression



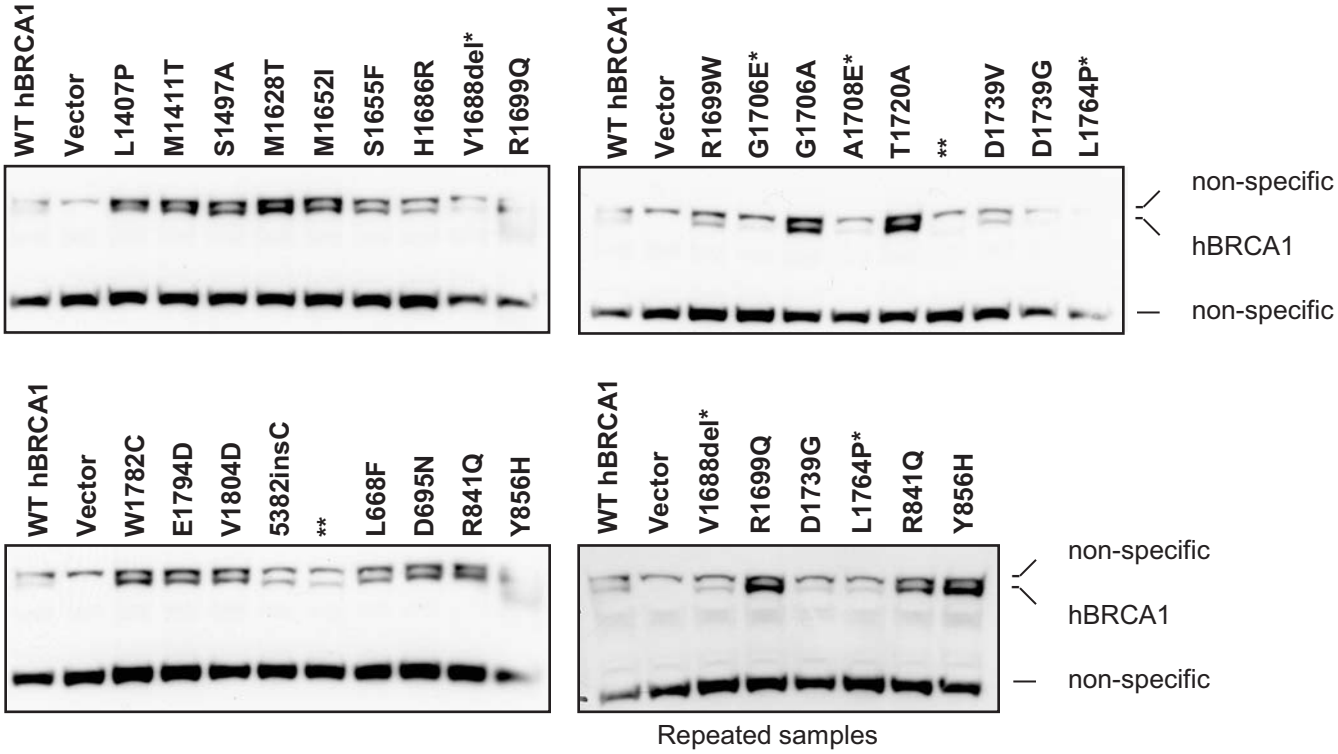
A: Introduction of the Frt and F3 sites for Flp RMCE into the wild-type *Rosa26* allele of *R26$^{CreERT2/wt}$;Brca1$^{SCo/\Delta}$* ES cells. E: EcoRI, X: XbaI, Nh: NheI. B: Southern blot with probe pHA607 on EcoRI digested DNA from *Brca1$^{SCo/\Delta}$* ES cells before and after targeting both *Rosa26* alleles with either *CreERT2* or Frt and F3 carrying vectors. Wild-type (15.6 kb), and *CreERT2* (5.9 kb) or RMCE cassette (4.3 kb) targeted *Rosa26* alleles are indicated for wild-type (lane 1) and *R26$^{CreERT2/RMCE}$;Brca1$^{SCo/\Delta}$* ES cells (lane 2). C: Western blot analysis of human BRCA1 protein expression in *R26^{CreERT2};Brca1$^{SCo/\Delta}$* ES cells complemented with human *BRCA1* by random integration of BAC RP11-812O5 (lane 1) compared to RMCE of human *BRCA1* cDNA (lane 2) or an empty RMCE vector (lane 3). Non-specific bands (*) and RNA pol II were used as loading controls.

Western blot analysis of BRCA1 VUS protein expression (classification series I-II)

Western blots series I: M18T, K45Q, D67Y, Y105C, P142H, L147F, L165P, 185delAG, S186Y, T231M, D245V, L246V, V271L, P798L, N810Y, T826K, R866C, S988A, M1006I, S1140G, E1214K, E1250K, E1282V, E1364K.

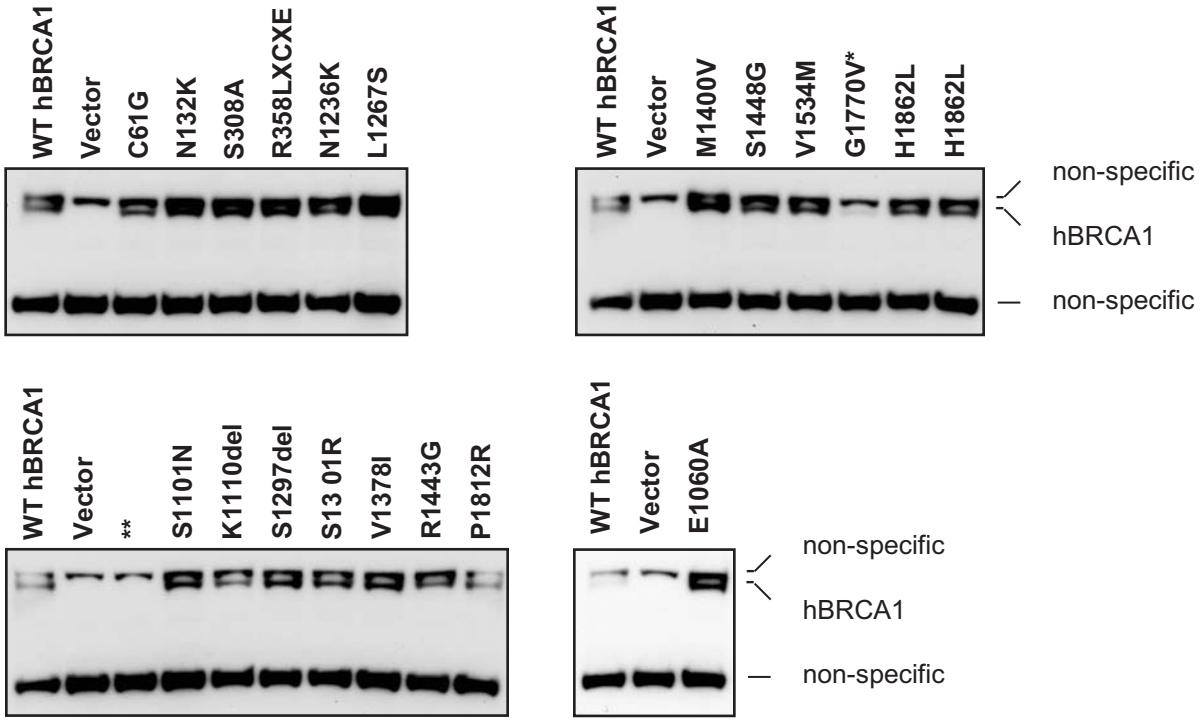


Western blots series II: L1407P, M1411T, S1497A, M1628T, M1652I, S1655F, H1686R, V1688del, R1699Q, R1699W G1706E, G1706A, A1708E, T1720A, D1739V, D1739G, L1764P, W1782C, E1794D, V1804D, 5382insC, L668F, D695N, R841Q, Y856H. Repeated: V1688del, R1699Q, D1739G, L1764P, R841Q, Y856H.

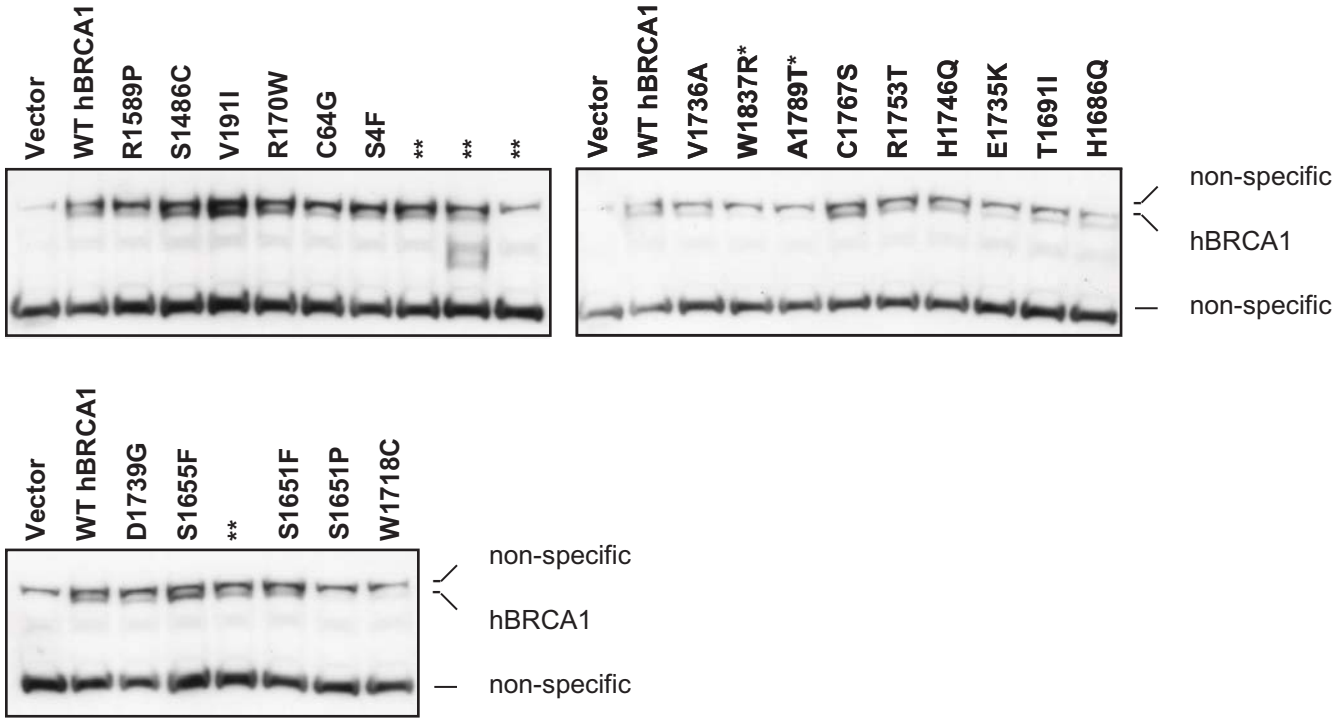


Western blot analysis of BRCA1 VUS protein expression (classification series III-IV)

Western blots series III: C61G, N132K, S308A, R358LXCXE, N1236K, L1267S, M1400V, S1448G, V1534M, G1770V, H1862L, S1101N, K1110del, S1297del, S1301R, V1378I, R1443G, P1812R, E1060A.

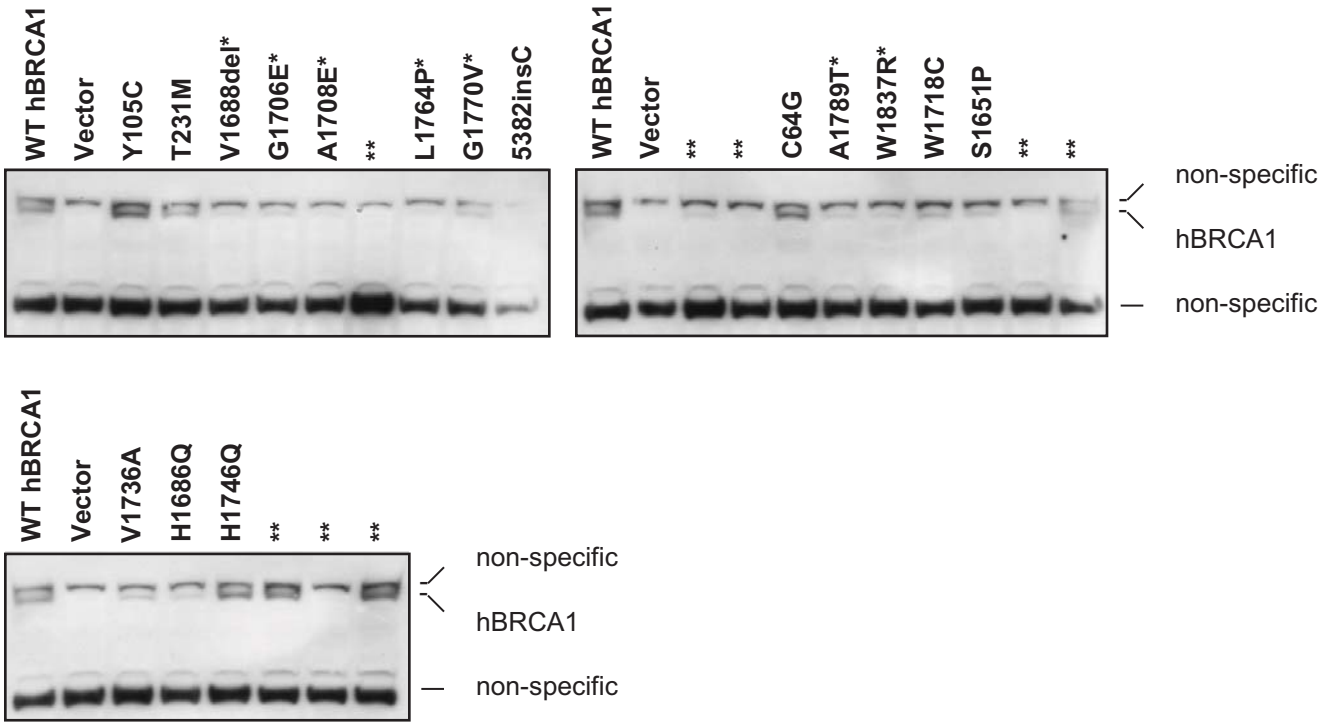


Western blots series IV: R1589P, S1486C, V191I, R170W, C64G, S4F, V1736A, W1837R, A1789T, C1767S, R1753T, H1746Q, E1735K, T1691I, H1686Q, D1739G, S1655F, S1651F, S1651P, W1718C.

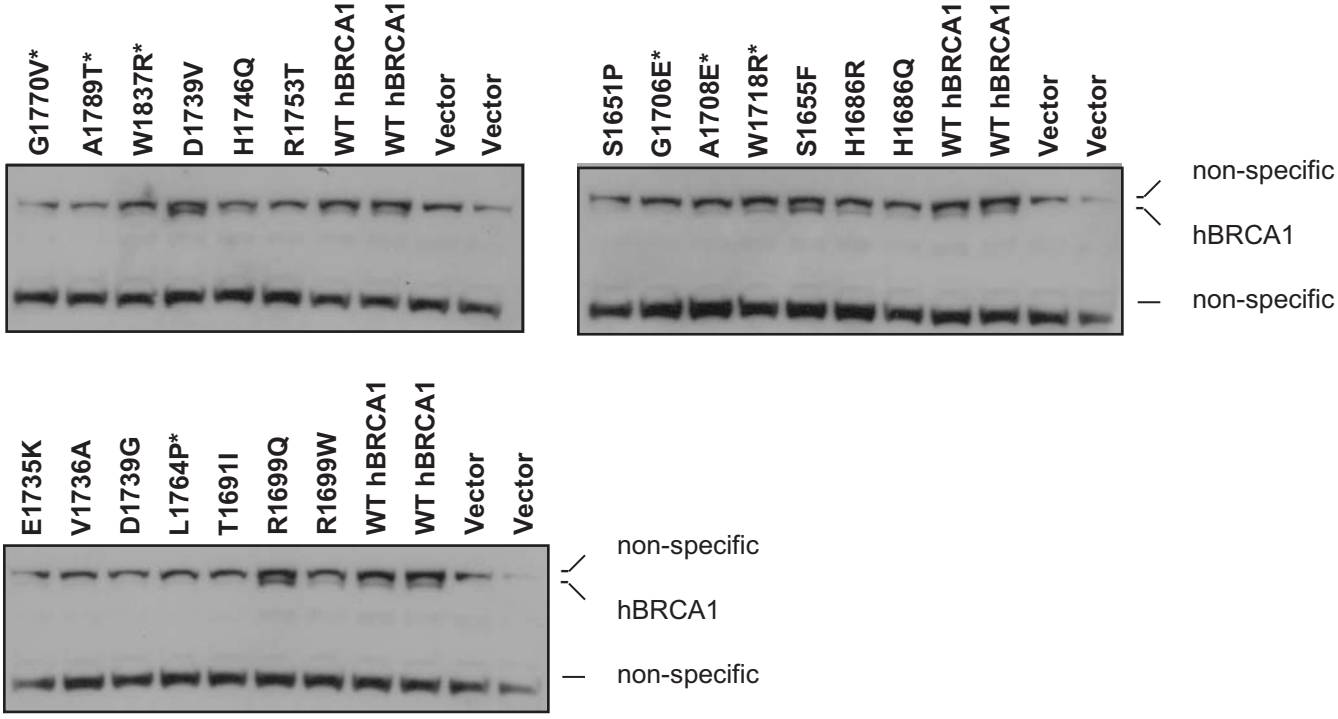


Western blot analysis of BRCA1 VUS protein expression (classification series V-VI)

Western blots series V: Y105C, T231M, V1688del, G1706E, A1708E, L1764P, G1770V, 5382insC, C64G, A1789T, W1738R, W1718C, S1651P, V1736A, H1686Q, H1746Q.

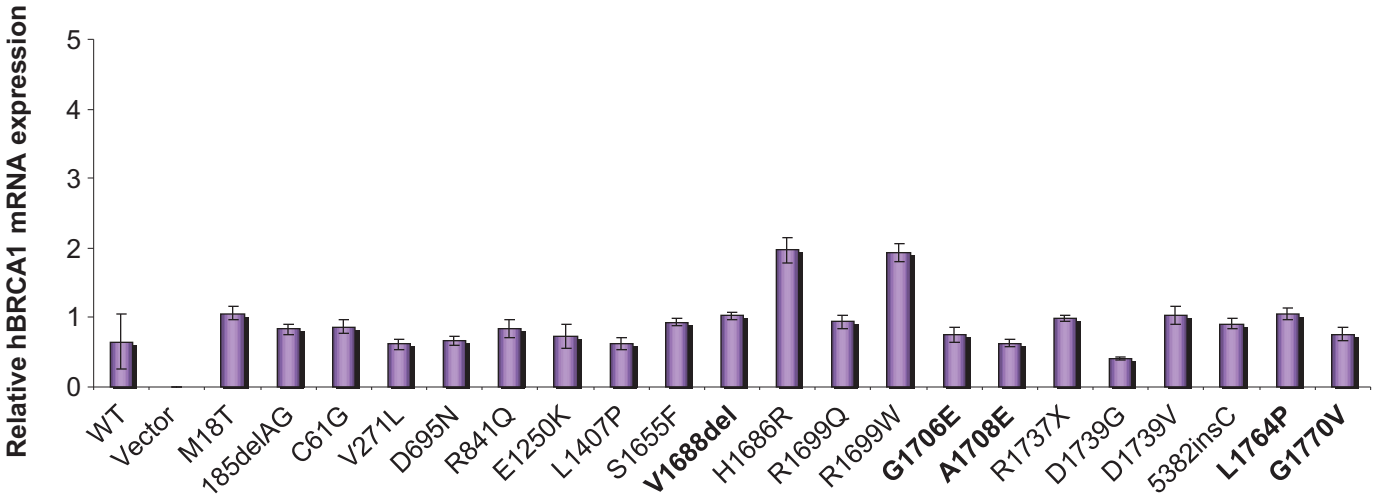


Western blots series VI: G1770V, A1789T, W1837R, D1739V, H1746Q, R1753T, S1651P, G1706E, A1708E, W1718R, S1655F, H1686R, H1686Q, E1735K, V1736A, D1739G, L1764P, T1691I, R1699Q, R1699W.



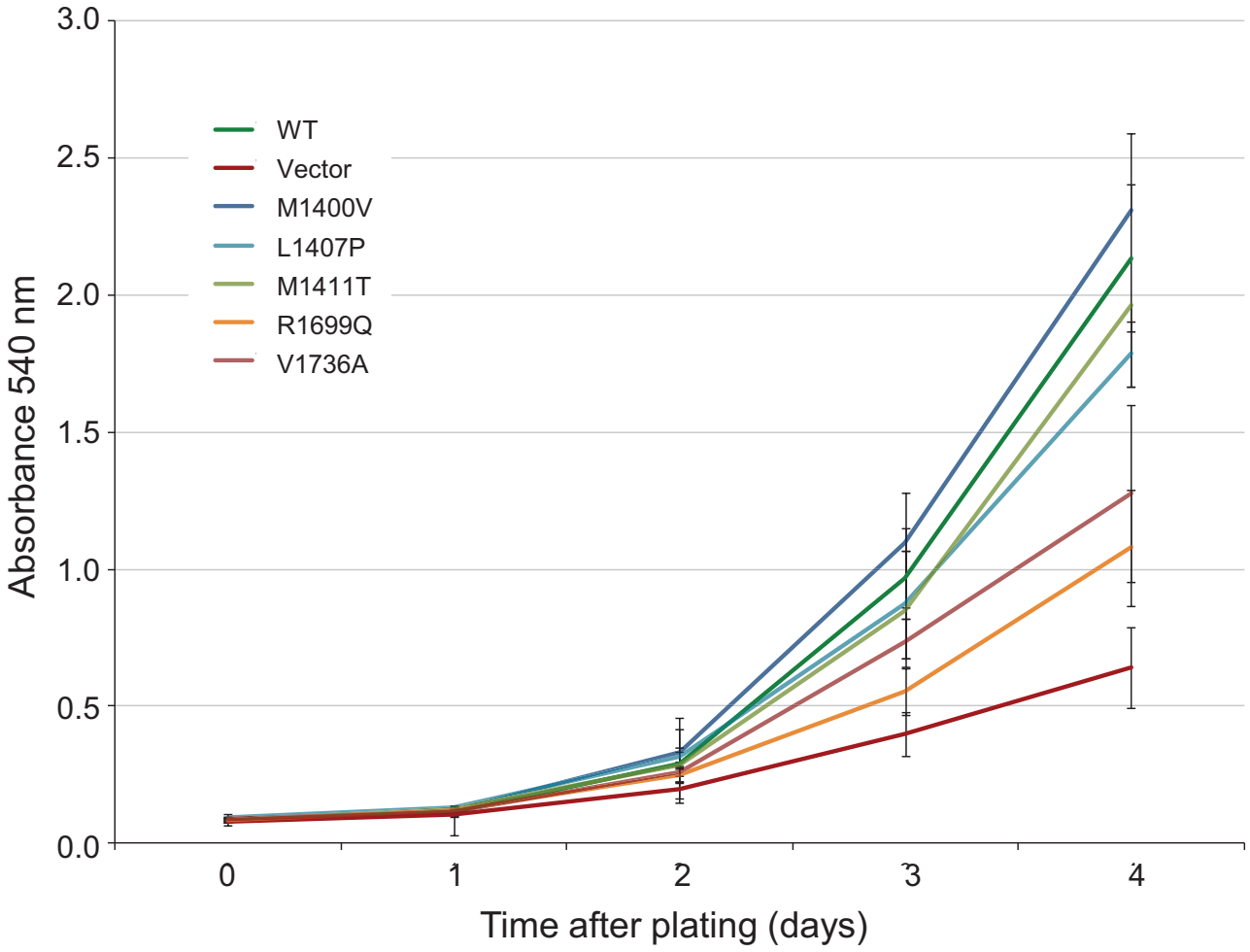
Western blot analysis of human (h) BRCA1 protein expression in *R26*$^{CreERT2/hBRCA1}$;*Brca1*$^{SCo/\Delta}$ ES cells using a polyclonal antibody (9010; Cell Signaling). *: BRCA1 variants which consistently showed low expression and the 185delAG mutant for which expression was validated by RT-PCR (Figure S3). ** BRCA1 variants which were not included in the classification assays. BRCA1 wild-type (WT) and empty RMCE vector (Vector) controls are present on each blot.

Real-time RT-PCR analysis of BRCA1 VUS mRNA expression



Real-time RT-PCR analysis of *BRCA1* VUS expression in *R26*$^{CreERT2/hBRCA1}$*; Brca1*$^{SCo/\Delta}$ ES cells was performed using primers specific for human *BRCA1* cDNA and normalized to *Rps20* expression. Experiments were performed in triplicate and including two independently transfected *BRCA1* wild-type (WT) and empty RMCE vector controls. Data were normalized to one of the WT controls and error bars indicate the standard deviation for two biological replicate experiments. VUS mutations resulting in low BRCA1 protein levels (Supplementary Table S2) are marked in bold.

Growth curves of BRCA1 VUS complemented ES cells generated in parallel with the PARP inhibitor sensitivity assay



Growth curves of mouse *Brca1*-deficient ES cells carrying *BRCA1* VUS or *BRCA1* wild-type (WT) or empty RMCE vector (Vector) controls in the *Rosa26* locus. $R26^{CreERT2/hBRCA1};Brca1^{SCo/\Delta}$ or $R26^{CreERT2/RMCE};Brca1^{SCo/\Delta}$ ES cells were switched using 4OHT and assayed for growth using SRB. Error bars indicate the standard deviation between the results of independently switched triplicate experiments.