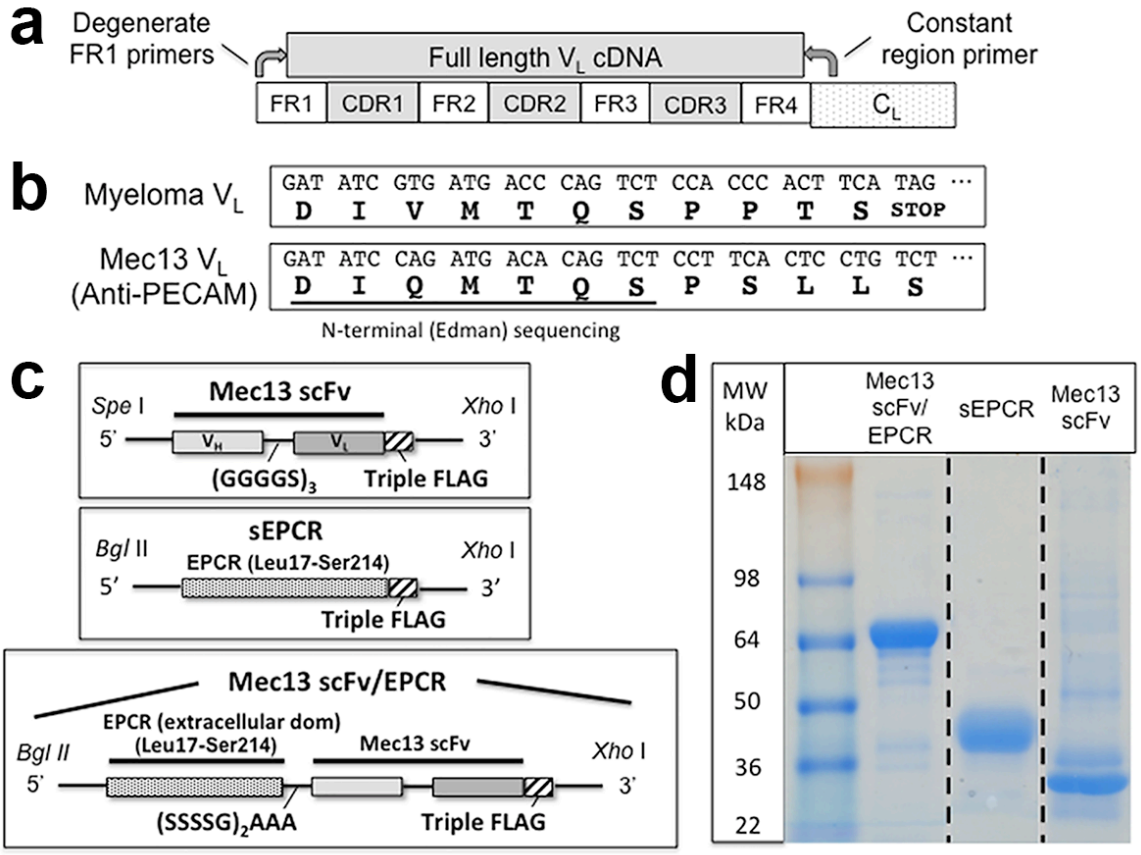
Supplemental Figure 1:



e

Protein	Target Molecule	Target Localization	⇧binding of 390 scFv/TM?	⇧APC by TM on REN cells?	⇧APC by 390 scFv/TM on ECs?
390 scFv/ TM	PECAM	Cell-cell borders	n/a	n/a	n/a
Mec13 scFv	PECAM	Cell-cell borders	yes	no	Yes (↑)
Mec13 scFv/EPCR	PECAM	Cell-cell borders	yes	yes	Yes (↑↑)
YN1 scFv/EPCR	ICAM	Apical membrane	no	yes	no

Supplemental Figure 1. Cloning, assembly, and purification of Mec13 scFv, sEPCR, and Mec13 scFv/EPCR fusion protein. (a) The typical approach to PCR cloning of variable heavy and light chain regions (V_H and V_L) utilizes degenerate 5' primers corresponding to the beginning of the FR1 region and a 3' primer corresponding to the start of the constant region. (b) In the case of the Mec13 hybridoma, the typical approach amplified only the myeloma V_L, which is nearly identical to the PECAM-specific V_L at the N-terminus of FR1 region. N-terminal (Edman) sequencing was used to identify a one amino acid difference (Gln vs. Val). Degenerate primers were synthesized and paired with the 3' constant region primer, enabling amplification of a full-length cDNA for the Mec13 V_L. (c) Assembly of V_H and V_L sequences into Mec13 scFv and Mec13 scFv/EPCR constructs. (d) SDS PAGE gel electrophoresis of Mec13 scFv, soluble EPCR, and Mec13 scFv/EPCR fusion protein shows relative size and purity. (e) Summary of key fusion proteins and scFv's, their surface targets, and their effects.