Supplementary Table 1

Sequence of major mesothelin isoform 622 amino acids

MALPTARPLLGSCGTPALGSLLFLLFSLGWVQPSRT
LAGETGQEAAPLDGVLANPPNISSLSPRQLLGFPCAEVSGLSTERVRELAVALAQKNVKLSTEQLRC
LAHRLSEPPEDLDALPLDLLLFLNPDAFSGPQACTRFFSRITKANVDLLPRGAPERQRLLPAALACW
GVRGSLLSEADVRALGGLACDLPGRFVAESAEVLLPRLVSCPGPLDQDQQEAARAALQGGGPPYG
PPSTWSVSTMDALRGLLPVLGQPIIRSIPQGIVAAWRQRSSRDPSWRQPERTILRPRFRR

**EVEKTACPSGKKAREIDESLIFYKKWELEACVDAALLATQMDRVNAIPFTYEQLDVLKHKLDELYP
QGYPESVIQHLGYLFLKMSPEDIRKWNVTSLETLKALLEVNKGHEMSPQVATLIDRFVKGRGQLD
KDTLDTLTAFYPGYLCSLSPEELSSVPPSSIWAVRPQDLDTCDPRQLDVLYPKARLAFQNMNGSEY
FVKIQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPLTVAEVQKLLGPHVEGLKAEERHR
PVRDWILRQRQDDLDTLGLGLQGGIPNGYLVLDLSMQEALS**GTPCLLGPGPVLTVLALLLASTLA*

1-36 signal seq
37-296 MPF
296-598 Membrane bound MSLN
599-622 GPI addition seq

Supplementary Table 2

Cleavage sites in mesothelin shed from A431/H9 cells

Sequence Number		Relative %
1	**^{568}QDDLDTLGLGLQGGIPN^{584}**	**29.0**
2	**^{568}QDDLDTLGLGLQGGIPNGY^{586}**	**11.0**
3	^{568}QDDLDTLGLGLQGGIPNGYL^{587}	2.0
4	^{568}QDDLDTLGLGLQGGIPNGYLV^{588}	1.0
5	^{568}QDDLDTLGLGLQGGIPNGYLVLD^{590}	2.0
6	**^{568}QDDLDTLGLGLQGGIPNGYLVLDL^{591}**	**54.0**
7	^{568}QDDLDTLGLGLQGGIPNGYLVLDLS^{592}	0.2
C-terminal Sequence	^{568}QDDLDTLGLGLQGGIPNGYLVLDLSVQEALS^{598}	

Supplementary Table 3

Cleavage sites in mesothelin from human ascites

Sequence Number		Relative %
1	^{568}QDDLDTLGL^{576}	5.7
2	^{568}QDDLDTLGLGL^{578}	7.1
3	^{568}QDDLDTLGLGLQ^{579}	2.2
4	**^{568}QDDLDTLGLGLQGGIPN^{584}**	**11.3**
5	^{568}QDDLDTLGLGLQGGIPNG^{585}	0.5
6	**^{568}QDDLDTLGLGLQGGIPNGY^{586}**	**10.3**
7	^{568}QDDLDTLGLGLQGGIPNGYL^{587}	5.8
8	^{568}QDDLDTLGLGLQGGIPNGYLV^{588}	1.6
9	^{568}QDDLDTLGLGLQGGIPNGYLVL^{589}	8.6
10	^{568}QDDLDTLGLGLQGGIPNGYLVLD^{590}	3.2
11	**^{568}QDDLDTLGLGLQGGIPNGYLVLDL^{591}**	**43.0**
12	^{568}QDDLDTLGLGLQGGIPNGYLVLDLSMQE^{595}	0.1
13	^{568}QDDLDTLGLGLQGGIPNGYLVLDLSMQEA^{596}	0.6
C-terminal Sequence	^{568}QDDLDTLGLGLQGGIPNGYLVLDLSMQEALS^{598}	

Supplementary Table 4

Supplemental Table 4. Interactions between antigen peptide and Fab of Mab 15B6

Peptide residue	Heavy chain residue	Distance (Å)	Light chain residue	Distance (Å)
Y586	Y54	3.5		
Y586	T30	2.63^		
Y586	S28	3.35^		
L587	None~			
V588	N52	3.5		
V588	Y33	3.64		
V588	Y54	3.67		
L589	None~			
D590	R50	2.69		
D590	H35	3.14		
D590	H_2O167	2.66	H_2O167-W98	2.94
L591	Y32	3.95		
L591	R98	3.59		
L591	E99	3.76		
S592	L100*	2.99		
S592			N36	2.80
S592			W98	3.63
M593			W93	3.53
M593			Y34	3.49
Q594			A51	3.71
Q594			N55	3.53
E595			Y34	2.82
A596			T53	3.69
A596			N54*	3.72/2.78
A596			T31	3.78
L597			N55	3.15
L597			N54*	2.92

^ Hydrogen bonding interaction

~ Residue side chain is not involved in contacting Fab

* Main chain atoms are involved in contact

Protein Data Bank (PDB) with accession codes 7U8C for the structure of 15B6/Peptide complex.

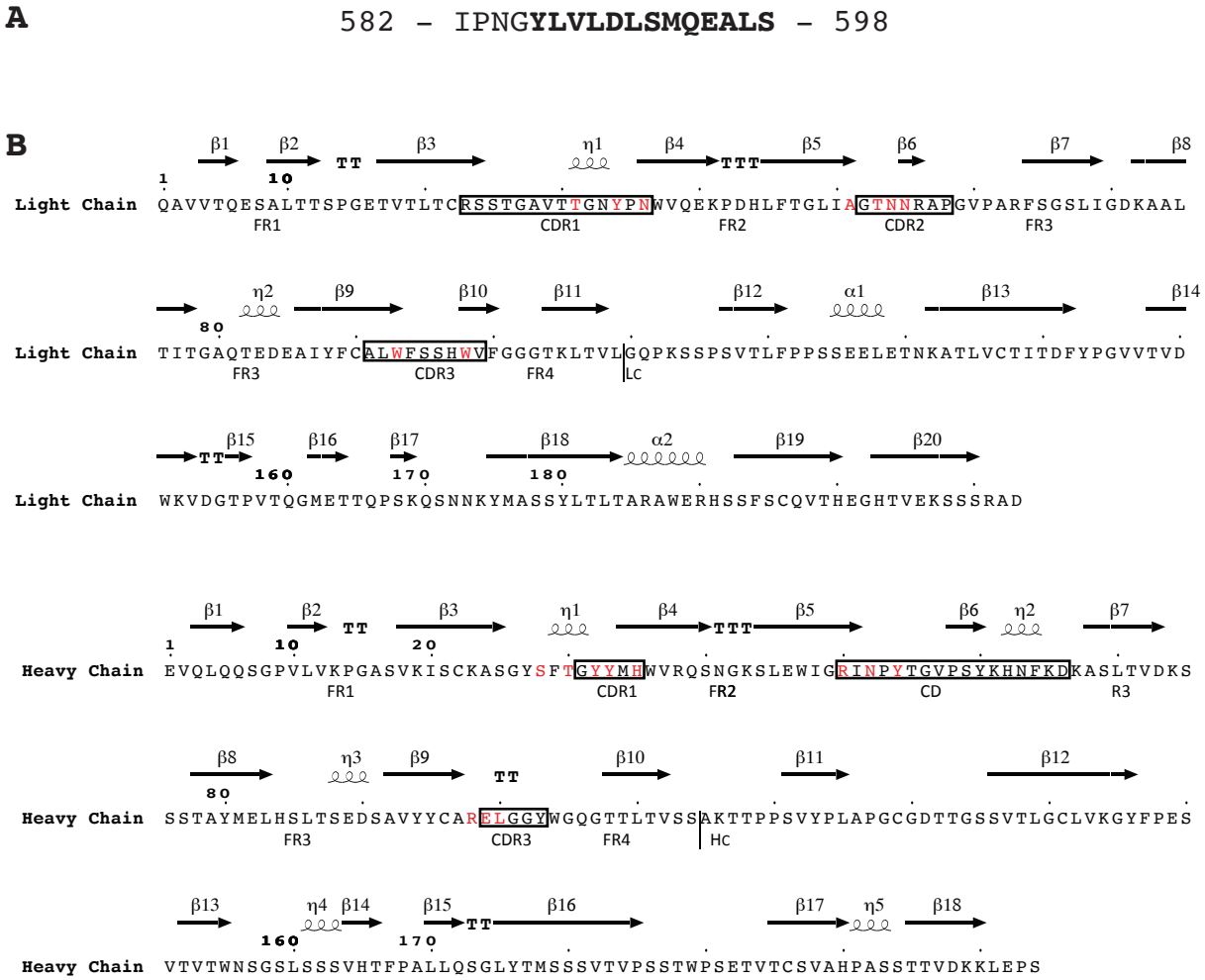
Supplementary Table 5

Mouse 15B6 amino acid sequence used in CAR-T

QAVVTQESALTTSPGETVTLTCRSSTGAVTTGNYPNWVQEKPDHLFTGLIAGTNNRAPGVPARFSGSLIGDKA
ALTITGAQTEDEAIYFCALWFSSHWVFGGGTKLTVLGGGGSGGGGSGGGGSEVQLQQSGPVLVKPGASVKIS
CKASGYSFTGYYMHWVRQSLVKRLEWIGRINPYTGVPSYKHNFKDKASLTVDKSSSTAYMELHSLTSEDSAVY
YCARELGGYWGQGTTLTVSS

Blue, VL sequence of 15B6
Red, linker sequence
Purple, VH sequence of 15B6

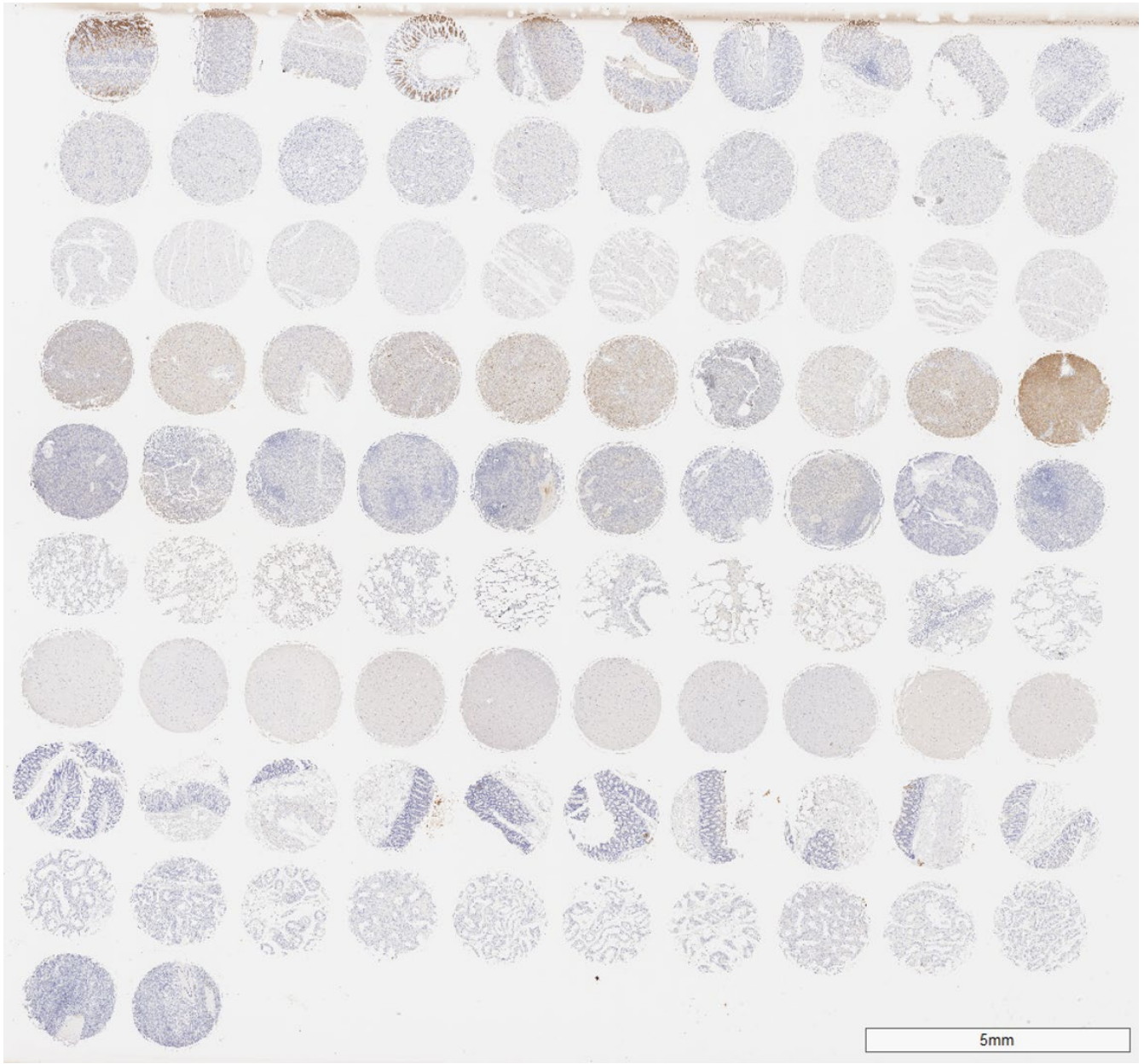
Supplementary Figure 1



Supplemental Figure 1. (A) The sequence of the peptide used in making the Fab/peptide complex. Residues that contact Fab are highlighted in boldface. (B) Sequences and secondary structure assignments of Fab light and heavy chains for Mab 15B6 are shown. Residue numbering is consistent with prior literature. CDRs are assigned according to the improved Chothia method and highlighted in boxes. The constant domains of the heavy and light chains are demarcated by a vertical line and indicated as CH and CL, respectively. The secondary structure elements are determined from the crystal structure and are annotated above the corresponding sequence with b-strands shown as arrows and a-helices as wiggly lines. Residues that make contacts with mesothelin peptide are colored in red.

Supplementary Figure 2

Tissue array showing 15B6 does not specifically bind to normal tissues



Stomach

Kidney

Heart

Liver

Spleen

Lung

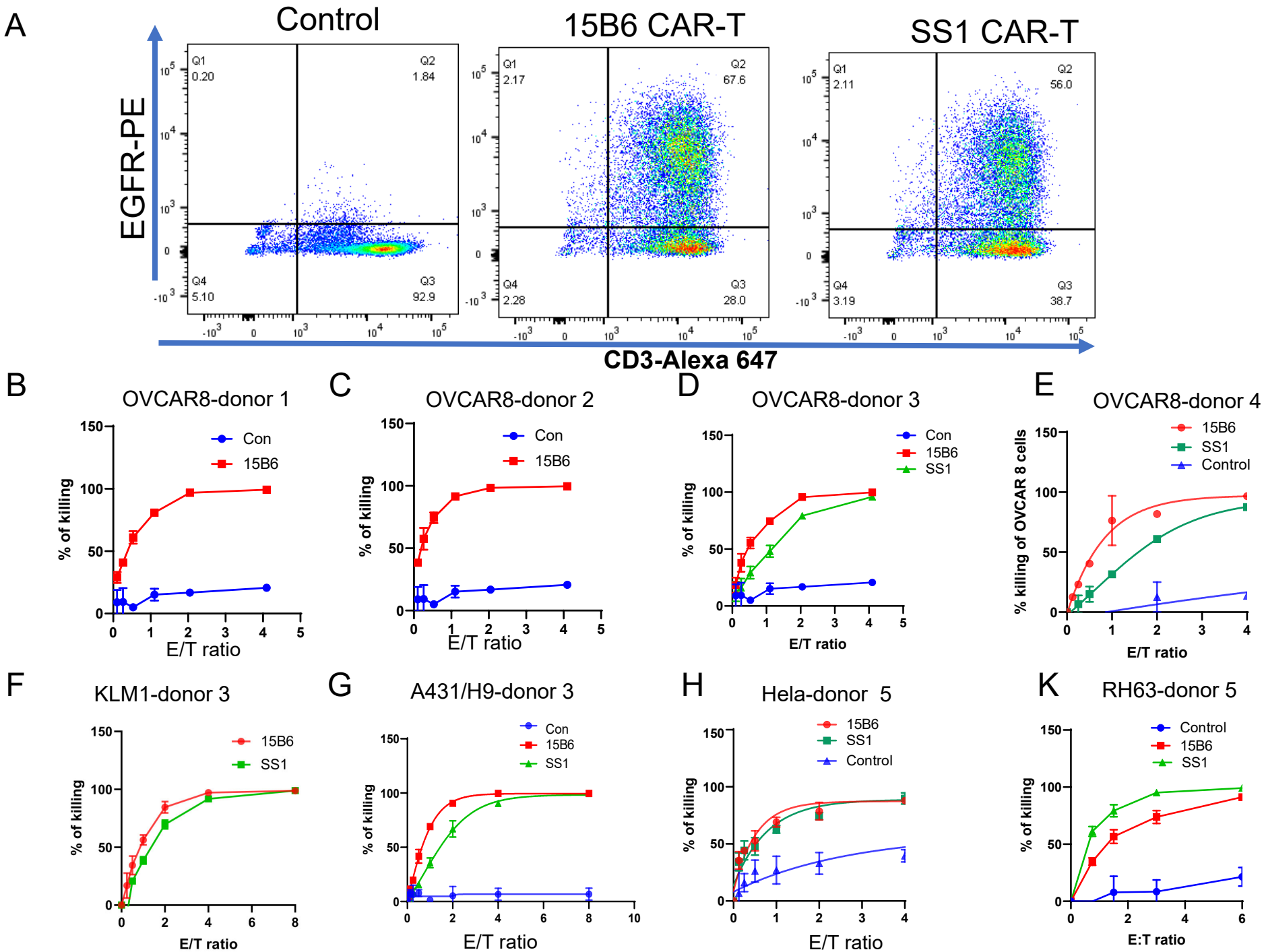
Brain

Colon

Testis

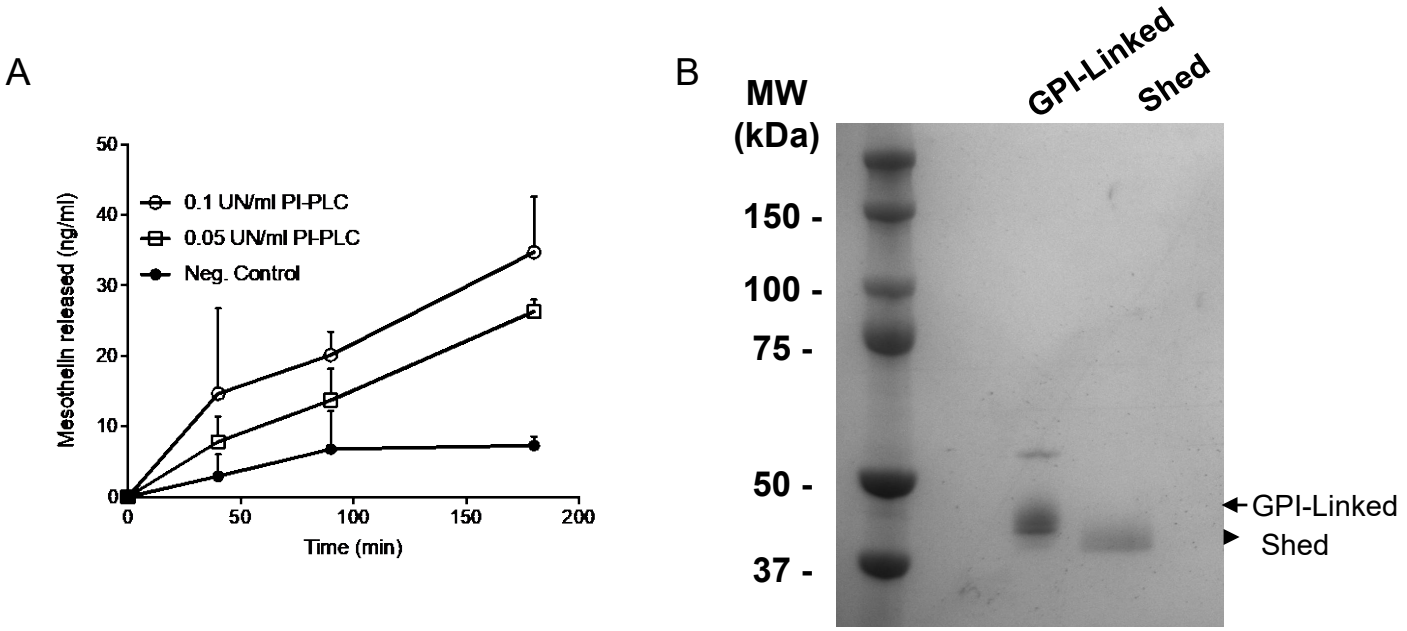
Ovary

Supplementary Figure 3



Supplementary Figure 3. **Transduction of CAR- T cells and its cytotoxicity tests in vitro.** A. Typical transduction efficiency of CAR-T cells. Lentivirus containing either 15B6 or SS1 CART vector were transduced into human PBMC. After 8 days of culture, the cells were stained with anti-CD3-Alexa647 and anti-EGFR-PE. Control is mock transduced cells. B-E. CAR-T cells made from 4 different donors were tested for the killing of OVCAR8-luc cells. F-H. CAR-T cells made with indicated donors were tested for the killing of cell lines, KLM1-luc (F), A431/H9-luc (G), Hela-luc (H) and primary mesothelioma line RH63-luc.

Supplementary Figure 4.



Generation of GPI-MSLN and comparison with shed MSLN A. Shed mesothelin concentrations were measured after treating A431/H9 cells with PI-PLC by Mesoscale assay. B. Non-reducing SDS-PAGE analysis comparing molecular weights of GPI-linked mesothelin and shed mesothelin.