

## Placenta-derived gp96 as a multivalent prophylactic cancer vaccine

Bao Zhao<sup>1,2,†</sup>, Yanzhong Wang<sup>1,†</sup>, Bo Wu<sup>1</sup>, Shan Liu<sup>3</sup>, Erjie Wu<sup>1</sup>, HongXia Fan<sup>1</sup>, MingMing Gui<sup>1</sup>, Lizhao Chen<sup>1</sup>, Changfei Li<sup>1</sup>, Ying Ju<sup>1</sup>, Wei Zhang<sup>3</sup>, Songdong Meng<sup>1,2,\*</sup>

1 CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences (CAS), Beijing, China

2 School of Life Sciences, University of Science and Technology of China, Hefei, China

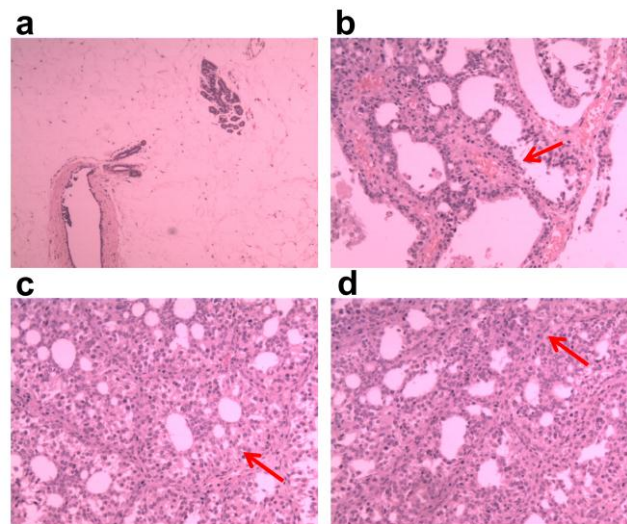
3 Detection Center of Tumor Biology, Cancer Institute & Hospital, Chinese Academy of Medical Sciences, Beijing, China

\* Corresponding author: Songdong Meng. CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, CAS, No.1 West Beichen Road, Chaoyang District, Beijing 100101, P. R. China.

Tel: (86)-10-64807350; Fax: (86)-10-64807351.

E-mail: [mengsd@im.ac.cn](mailto:mengsd@im.ac.cn)

† These authors contributed equally to this work.



Supplementary Fig. S1 Mammary tumor histology. (a) Paraffin-embedded breast tissue sections from a P-gp96 immunized rat. (b-d) Paraffin-embedded breast tissue sections from unimmunized (PBS) rats. Intraductal proliferation (IDP) (b); ductal carcinoma *in situ* (DCIS) (c); and invasive ductal carcinoma (IDC) (d). Original magnification,  $\times 40$ .

Supplementary Table. S1. Characteristics of DMBA-induced mammary tumors in PBS- and P-gp96-immunized female Wistar rats.

<b>Characteristic</b>	<b>PBS (n=7)</b>	<b>P-gp96 (n=5)</b>
Final body weight (g)	227 ±3.29	295 ±7.5
Tumor onset		
Day of first tumor <sup>a</sup>	91	No tumors present <sup>c</sup>
Tumor incidence (%)	7/7 (100)	0/5 (0 <sup>c</sup> )
Tumor volume (mm <sup>3</sup> )	6262.63 ±323.20	0 <sup>c</sup>
Mean tumor weight (g)	14.12	0 <sup>c</sup>
Multiplicity	7.4 ±2.1	0 <sup>c</sup>
Total tumors <sup>b</sup>	52	0 <sup>c</sup>
IDP	17	0
DCIS	17	0
IDC	18	0

<sup>a</sup> Post-DMBA day at which the first mammary tumor was detectable by palpation. No tumors were detected in the gp96 immunized rats by the end of the experiment. <sup>b</sup> Number of total mammary tumors graded as IDP, DCIS, or IDC. <sup>c</sup> *P*<0.001.

Supplementary Table S2. Primers for Real-time PCR.

Mouse MUC1	Forward: ATCGTCTATTTCCCTTGCCCTG Reverse: TCCTACAAGTTGGCAGAAGTG
Mouse HER2	Forward: CCCTCATCACCTACAACACAG Reverse: CAGACCATAGCATACTCCAGC
Mouse MMP2	Forward: GTCTTCCCCTTCACTTTCCCTG Reverse: CGGAAGTTCTTGGTGTAGGTG
Mouse FGF2	Forward: GGAGTTGTGTCTATCAAGGGAG Reverse: CAGTATGGCCTTCTGTCCAG
Mouse c-Myc	Forward: CTTTCCCTACCCGCTCAAC Reverse: CTTCTCCACAGACACCACATC
Mouse VEGF-a	Forward: GAGAGCAGAAGTCCCATGAAG Reverse: GCTTTGGTGAGGTTTGTATCC
Mouse β-actin	Forward: ACCGTGAAAAGATGACCCAG Reverse: GAGCATAGCCCTCGTAGATG

Mouse GAPDH	Forward: AAATTCAACGGCACAGTCAAG Reverse: GTTCACACCCATCACAAACATG
Rat MUC1	Forward: TGGCTTTGGTCATCGTCTATC Reverse: CCTGTGCGAAACCTCTTCATAGG
Rat HER2	Forward: TTTGGCACTGTCTACAAGGG Reverse: ACACCAGCCATCACATACG
Rat MMP2	Forward: TGAGAAGGATGGCAAGTATGG Reverse: TGTTGCCAGGAAAGTGAAG
Rat FGF2	Forward: TCTACTGCAAGAACGGCG Reverse: TCGTTTCAGTGCCACATACC
Rat c-Myc	Forward: CTCGCGTTATTTGAAGCCTG Reverse: CAACATAGGACGGAGAGCAG
Rat VEGF-a	Forward: GAGTACCCCGATGAGATAGAGT Reverse: TTTGACCCTTTCCCTTTCCTC
Rat $\beta$ -actin	Forward: CAACTGGGACGATATGGAGAAG Reverse: CAGAGGCATACAGGGACAAC
Rat GAPDH	Forward: ACAATGCCTGGATCCCTAAAG Reverse: CACACCGACCTTCACCATC
Human MUC1	Forward: TGGTCTGTGTTCTGGTTGC Reverse: CCCTACAAGTTGGCAGAAGTG
Human HER2	Forward: GAGACCCGCTGAACAATACC Reverse: CTTACACATCGGAGAACAGGG
Human MMP2	Forward: GAGTTGGCAGTGCAATACCT Reverse: GCCGTCCTTCTCAAAGTTGT
Human FGF2	Forward: CTGGCTATGAAGGAAGATGGA Reverse: TGCCCAGTTCGTTTCAGTG
Human c-Myc	Forward: TCAAGAGGCGAACACACAAC Reverse: GGCCTTTTCATTGTTTTCCA
Human VEGF-a	Forward: ATGACGAGGGCCTGGAGTGTG Reverse: CCTATGTGCTGGCCTTGGTGAG
Human $\beta$ -actin	Forward: GCACTCTTCCAGCCTTCC Reverse: GTGATCTCCTTCTGCATCCTG
Human GAPDH	Forward: GTCCACTGGCGTCTTCAC Reverse: GAGTCCTTCCACGATACCAAAG