

SUPPLEMENTARY DATA:

FIGURE 1. Western blot analysis of CTR knock-down in PC-3M cells. PC-3M cells were stable transfected with plasmid pRNA-H1.1/Hygro (Genescript, Piscataway, NJ) containing previously validated siRNA duplexes against CTR mRNA (17,23). Six weeks after selection in hygromycin (400 μ g/ml), the cell clones were grown and expanded. CTR expression in the cell lines was checked by Western blot analysis. Vector control contained scrambled siRNA duplexes of equivalent length.

V. Vector Control; 1. Clone 1; 2. Clone 2.

FIGURE 2. Adhesion characteristics of three clones of each PC subline used in the present study. A. Compaction pattern (100X) of subconfluent cultures of three different clones of each cell lines used in Figure 1: a) empty vector transfected PC-3M cells (PC-3M-V); b) CT down-regulated PC-3M cells (PC-3M-CT⁻); c) CTR down-regulated PC-3M cells (PC-3M-CTR⁻); d) PC-3 cells transfected with empty vector (PC-3-V); e) PC-3 cells with enforced CTR (PC-3-CTR). B. Formation of acinus structure in 3-D collagen gel—Three separate clones of each PC cell line used in Figure 1C were tested for acinus formation in 3D culture as described in Figure 1C. a) LNCaP vector controls (LNCaP-V); b) LNCaP cells with enforced CT expression (LNCaP-CT); c) PC-3M vector controls (PC-3M-V); d) CT down-regulated PC-3M cells (PC-3M-CT⁻); e) CTR down-regulated PC-3M cells (PC-3M-CTR⁻). C. Three separate clones of PC-3M-V, PC-3M-CTR⁻, PC-3-V and PC-3-CTR cells were grown on Transwells, and allowed to polarize for 3 days after plating. TER was measured after 3 days. The results are mean TER \pm SEM (n=3). Data were compiled from three separate experiments.

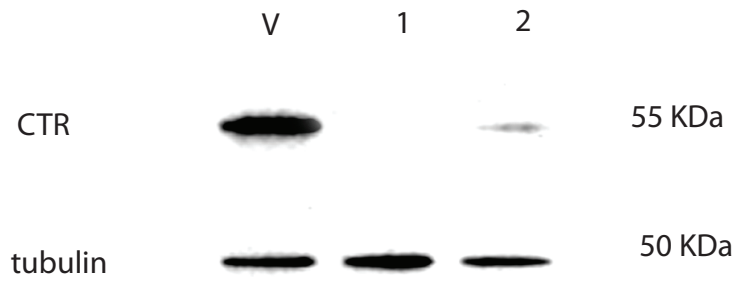
TABLE 1: Effect of CT (50 nM) on WNT signaling pathway-related gene expression in PC-31-CTR cells as assessed in WNT signaling pathway Oligo GEArray membrane (OHS-043).

Supplementary Table 1: Effect of CT on WNT signaling pathway related gene expression in PC-31-CTR cells

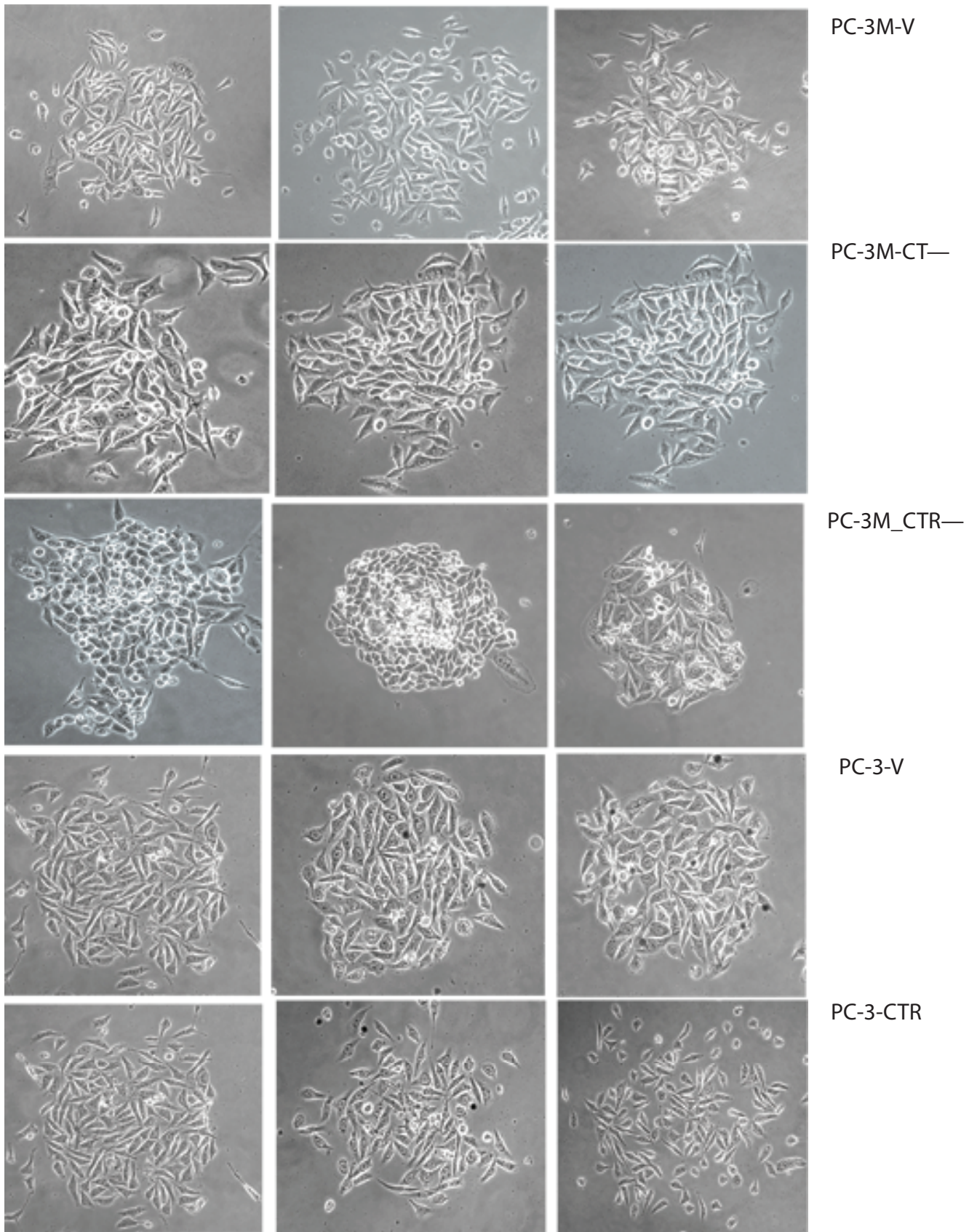
| | V | CT (50 nM) | mPKI (200 nM) | mPKI+CT |
|--------|---|------------|---------------|---------|
| WNT1 | + | +++ | + | + |
| WNT2 | | | | |
| WNT2B | | | | |
| WNT3 | + | ++ | + | + |
| WNT3A | | | | |
| WNT4 | | | | |
| WNT5A | | | | |
| WNT5B | | | | |
| WNT6 | | | | |
| WNT7A | | | | |
| WNT7B | | | | |
| WNT8A | | | | |
| WNT8B | | | | |
| WNT9A | | | | |
| WNT9B | | | | |
| WNT10A | | | | |
| WNT10B | | | | |
| WNT11 | | | | |
| WNT16 | | | | |
| FZD1 | + | ++ | + | ++ |
| FZD2 | + | +++ | + | ++ |
| FZD3 | | | | |
| FZD4 | | | | |
| FZD5 | | | | |
| FZD6 | | | | |
| FZD7 | | | | |
| FZD8 | + | ++ | + | + |
| FZD9 | | | | |
| FZD10 | + | ++ | + | ++ |
| LRP5 | | | | |
| LRP6 | | + | | |
| CTNNB1 | | ++ | | |
| APC | | | | |
| APC2 | | | | |
| AXIN1 | | + | | |
| AXIN2 | | | | |
| DVL1 | | | | |
| DVL2 | | | | |
| DVL3 | | + | | |
| GSK3A | | | | |
| GSK3B | | | | |
| LEF1 | | ++ | | |
| TCF7 | | ++ | | |
| TCF7L2 | | ++ | | |

Expression was evaluated as follows: +-low; ++-intermediate; +++-high.

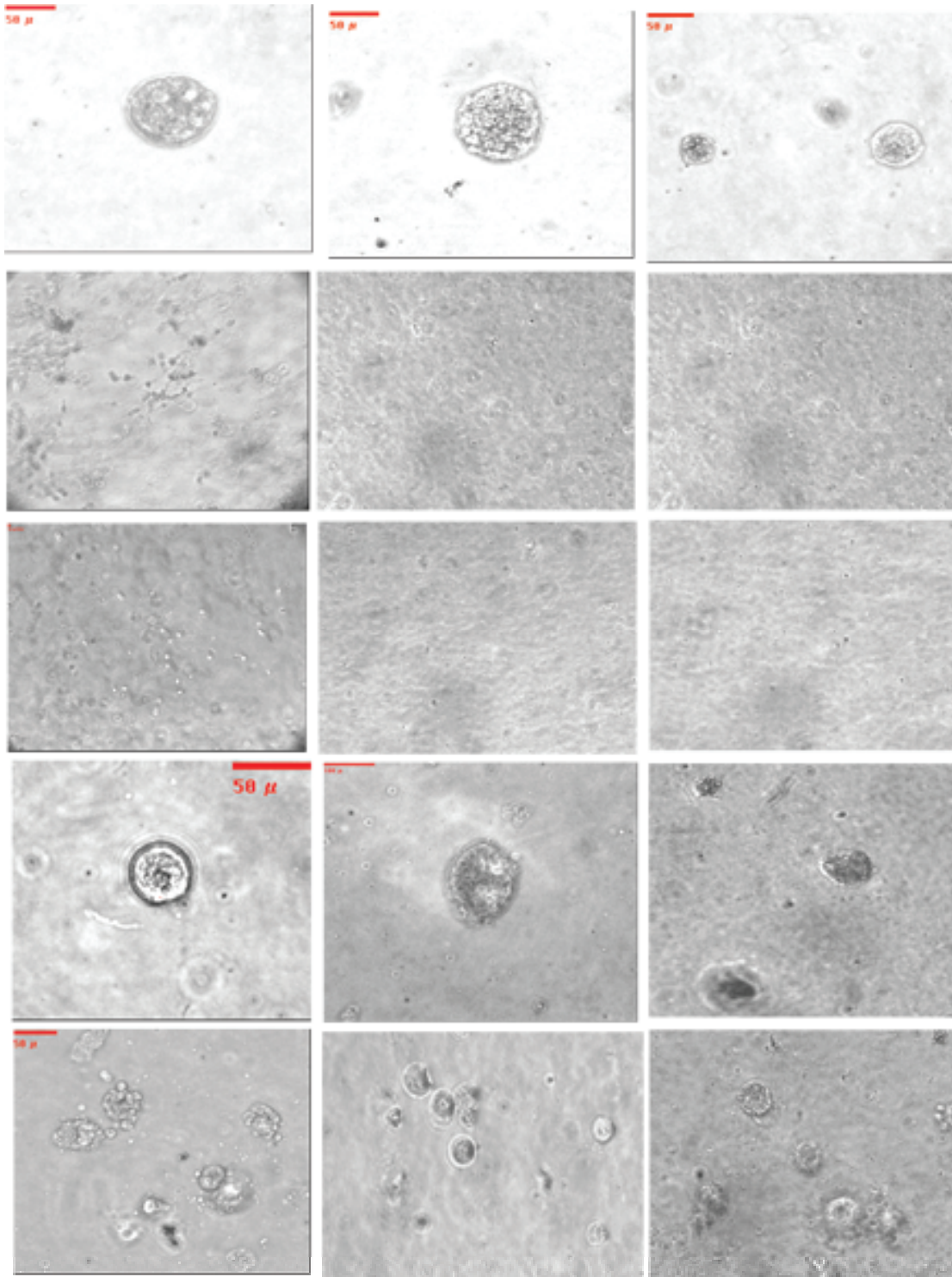
Supplementary Figure 1



A.



B.



LNCaP-V

LNCaP-CT

PC-3M-V

PC-3M-CT

PC-3M-CTR

C.

