## LRP5 regulates the expression of STK40, a new potential target in triple-negative breast cancers

## SUPPLEMENTARY MATERIALS

|                  |      | Tumor volume (mm3) |        |        |        |        |        |        |  |  |  |
|------------------|------|--------------------|--------|--------|--------|--------|--------|--------|--|--|--|
|                  | mice | Day 8              | Day 13 | Day 16 | Day 20 | Day 23 | Day 27 | Day 30 |  |  |  |
| control<br>siRNA | 1    | 40                 | 87.5   | 87.5   | 144    | 144    | 196    | 220.5  |  |  |  |
|                  | 2    | 32                 | 75     | 126    | 162    | 269.5  | 416    | 416    |  |  |  |
|                  | 3    | 40                 | 48     | 75     | 87.5   | 126    | 196    | 245    |  |  |  |
|                  | 4    | 0                  | 0      | 62.5   | 75     | 126    | 162    | 288    |  |  |  |
|                  | 5    | 22.5               | 40     | 62.5   | 126    | 144    | 144    | 144    |  |  |  |
|                  | 6    | 13.5               | 32     | 62.5   | 75     | 75     | 112.5  | 125    |  |  |  |
|                  | 7    | 22.5               | 48     | 75     | 108    | 126    | 196    | 196    |  |  |  |
| LRP5<br>siRNA    | 1    | 18                 | 32     | 32     | 32     | 32     | 32     | 62.5   |  |  |  |
|                  | 2    | 0                  | 32     | 40     | 62.5   | 62.5   | 62.5   | 75     |  |  |  |
|                  | 3    | 0                  | 62.5   | 75     | 126    | 126    | 196    | 288    |  |  |  |
|                  | 4    | 40                 | 40     | 62.5   | 108    | 75     | 108    | 126    |  |  |  |
|                  | 5    | 0                  | 75     | 108    | 126    | 126    | 196    | 196    |  |  |  |
|                  | 6    | 32                 | 75     | 75     | 126    | 126    | 171.5  | 256    |  |  |  |
|                  | 7    | 0                  | 40     | 62.5   | 75     | 75     | 75     | 75     |  |  |  |
| LRP6<br>siRNA    | 1    | 0                  | 32     | 40     | 48     | 48     | 87.5   | 144    |  |  |  |
|                  | 2    | 0                  | 0      | 0      | 0      | 40     | 87.5   | 126    |  |  |  |
|                  | 3    | 40                 | 40     | 62.5   | 62.5   | 75     | 87.5   | 144    |  |  |  |
|                  | 4    | 0                  | 0      | 40     | 40     | 62.5   | 62.5   | 75     |  |  |  |
|                  | 5    | 40                 | 40     | 40     | 40     | 62.5   | 87.5   | 87.5   |  |  |  |
|                  | 6    | 0                  | 0      | 40     | 40     | 40     | 40     | 40     |  |  |  |
|                  | 7    | 62.5               | 62.5   | 40     | 75     | 126    | 126    | 162    |  |  |  |
| STK40<br>siRNA   | 1    | 40                 | 40     | 62.5   | 108    | 126    | 126    | 126    |  |  |  |
|                  | 2    | 18                 | 40     | 40     | 75     | 62.5   | 126    | 126    |  |  |  |
|                  | 3    | 32                 | 32     | 62.5   | 62.5   | 108    | NA     | NA     |  |  |  |
|                  | 4    | 32                 | 40     | 40     | 75     | 75     | 108    | 108    |  |  |  |
|                  | 5    | 32                 | 32     | 32     | 40     | 62.5   | 75     | 75     |  |  |  |
|                  | 6    | 0                  | 75     | 75     | 108    | 126    | 196    | 256    |  |  |  |
|                  | 7    | 40                 | 40     | 40     | 40     | 72     | 87.5   | 126    |  |  |  |

|        | LRP5      |           | LF        | RP6       | STK40     |           |
|--------|-----------|-----------|-----------|-----------|-----------|-----------|
|        |           | square    |           | square    |           | square    |
|        | raw       | rooted    | raw       | rooted    | raw       | rooted    |
|        | (adjusted | (adjusted | (adjusted | (adjusted | (adjusted | (adjusted |
|        | P value)  |
| Day 8  | 0.782     | 0.152     | 0.889     | 0.301     | 0.889     | 0.831     |
| Day 13 | 0.889     | 0.615     | 0.532     | 0.101     | 0.889     | 0.875     |
| Day 16 | 0.747     | 0.584     | 0.168     | 0.047     | 0.385     | 0.236     |
| Day 20 | 0.657     | 0.566     | 0.015     | 0.006     | 0.195     | 0.171     |
| Day 23 | 0.052     | 0.096     | 0.003     | 0.015     | 0.054     | 0.110     |
| Day 27 | 0.003     | 0.032     | 0.000     | 0.003     | 0.003     | 0.047     |
| Day 30 | 0.003     | 0.047     | 0.003     | 0.004     | 0.001     | 0.032     |

**Supplementary Figure 1: Tumor volumes and statistical analysis of the in vivo experiment (see Figure 7). Top table:** tumor volume (mm³) determined for each mice following injection with siRNA transfected MDA-MB-468 at different time points (day 8, day 13, day 16, day 20, day 23, day 27, day 30). NA: not applicable (the mouse n°3 of the STK40 group died during the experiment). **Bottom table:** Statistical analysis of the xenograft experiment. We considered an anova model with 28 groups (7 days of tumor measurement, 4 different siRNA) and tested the difference between the control siRNA and the other siRNAs (LRP5, LRP6 or STK40) at each time point (a total of 21 tests) (columns "raw"). To take into account that variances were higher for larger measurements, we also ran the anova model on the square-rooted data (columns "square rooted"). We adjusted for multiple testing using the Benjamini Hochberg correction. Differences were considered significant if the adjusted *P* value was below 0.05 (indicated in bold and red).

**Supplementary Table 1: Analysis of gene expression in HCC38 cells depleted of LRP5 and LRP6.** This table lists the genes specifically up- or downregulated by LRP5 or LRP6 depletion, and the genes displaying a change in expression following the depletion of both receptors 24 h after transfection with siRNA (fold change > 1.3 relative to the control siRNA). STK40 was one of two genes specifically downregulated by LRP5 depletion.

See Supplementary File 1