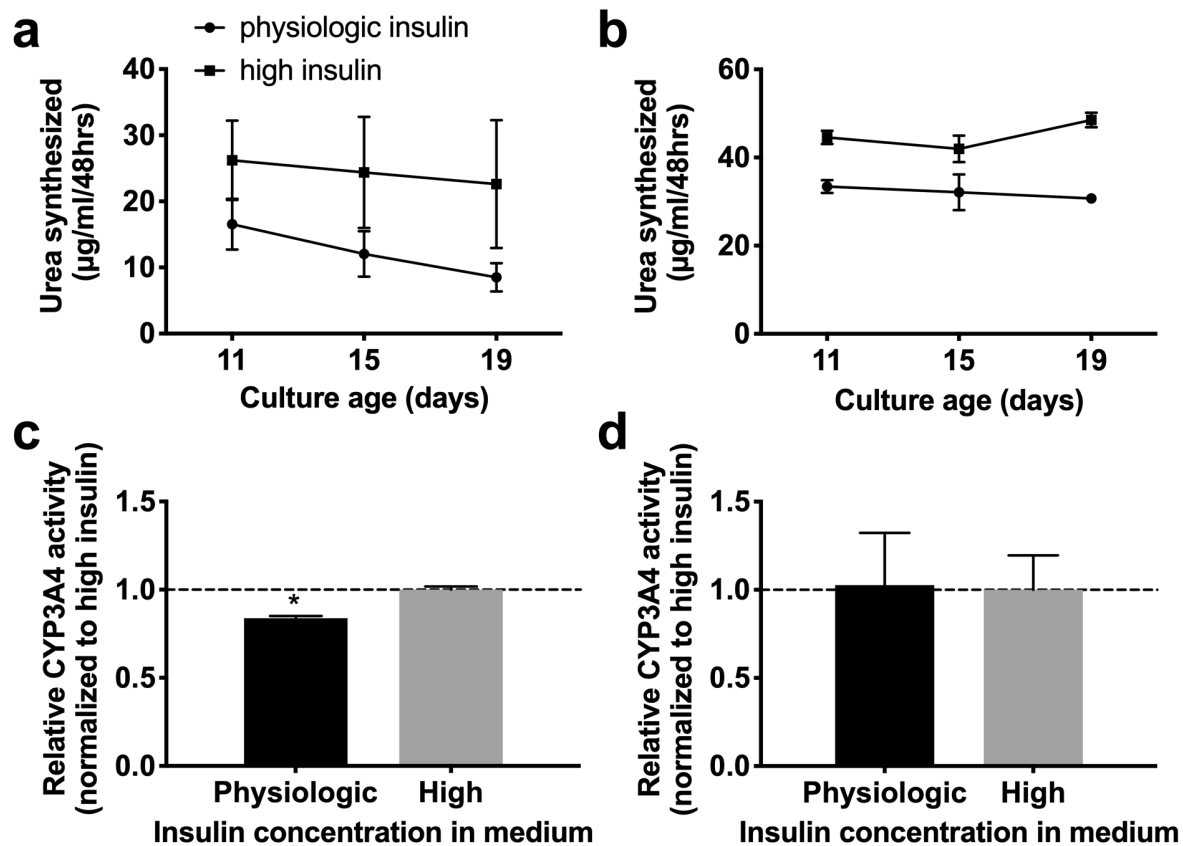


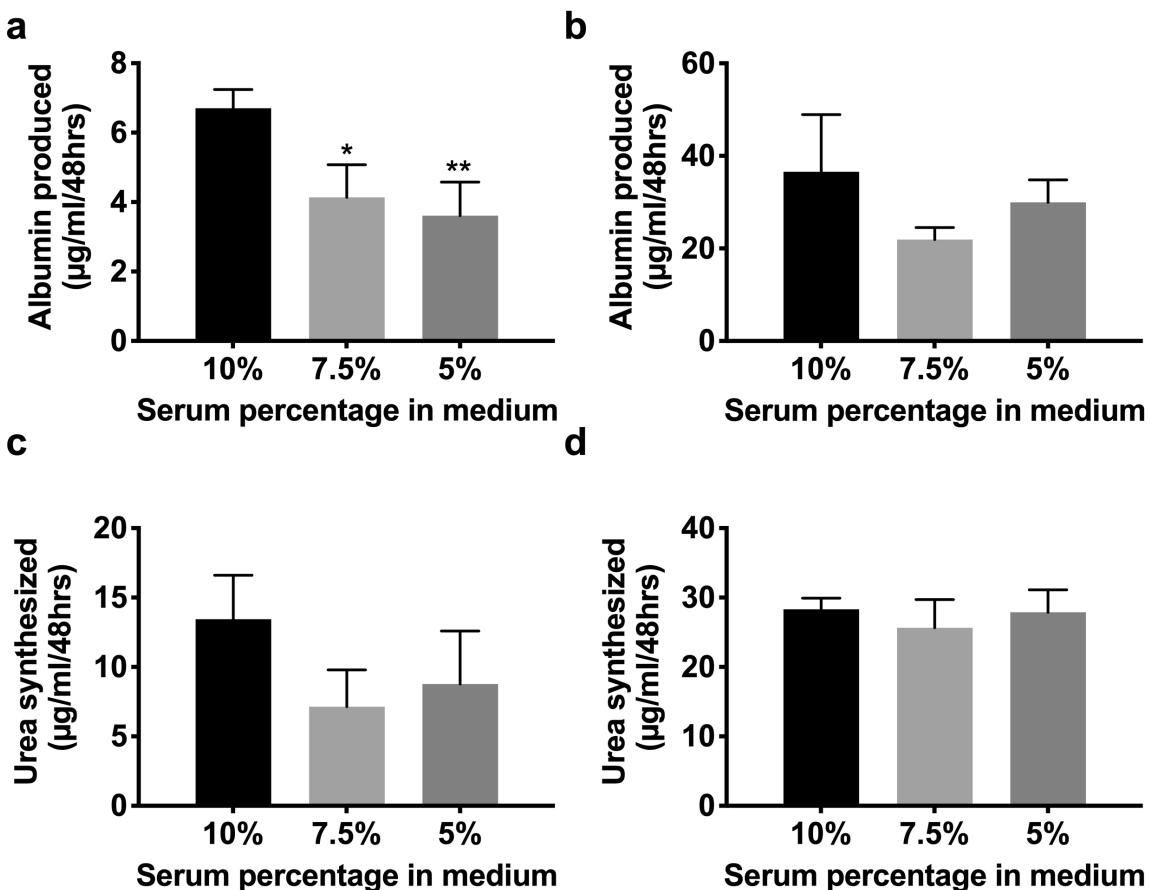
## SUPPLEMENTAL DATA

### Physiologically Inspired Culture Medium Prolongs the Lifetime and Insulin Sensitivity of Human Hepatocytes in Micropatterned Co-cultures

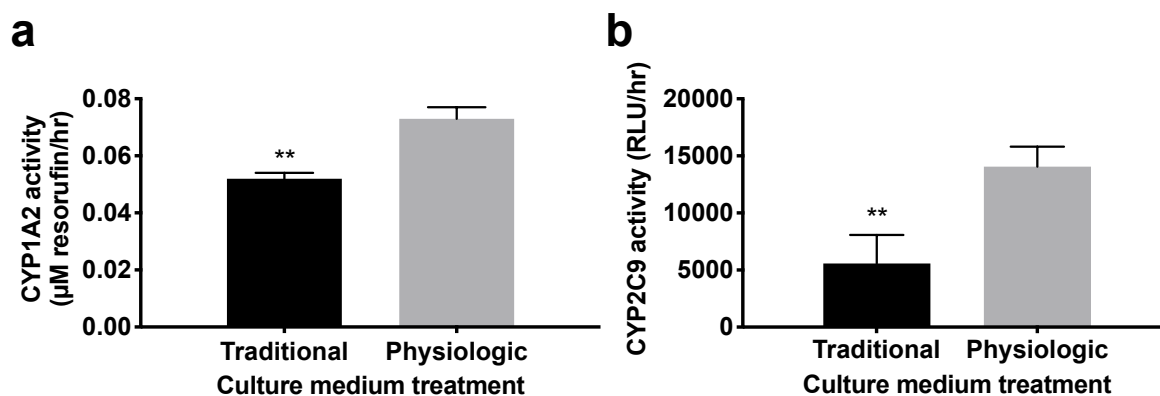
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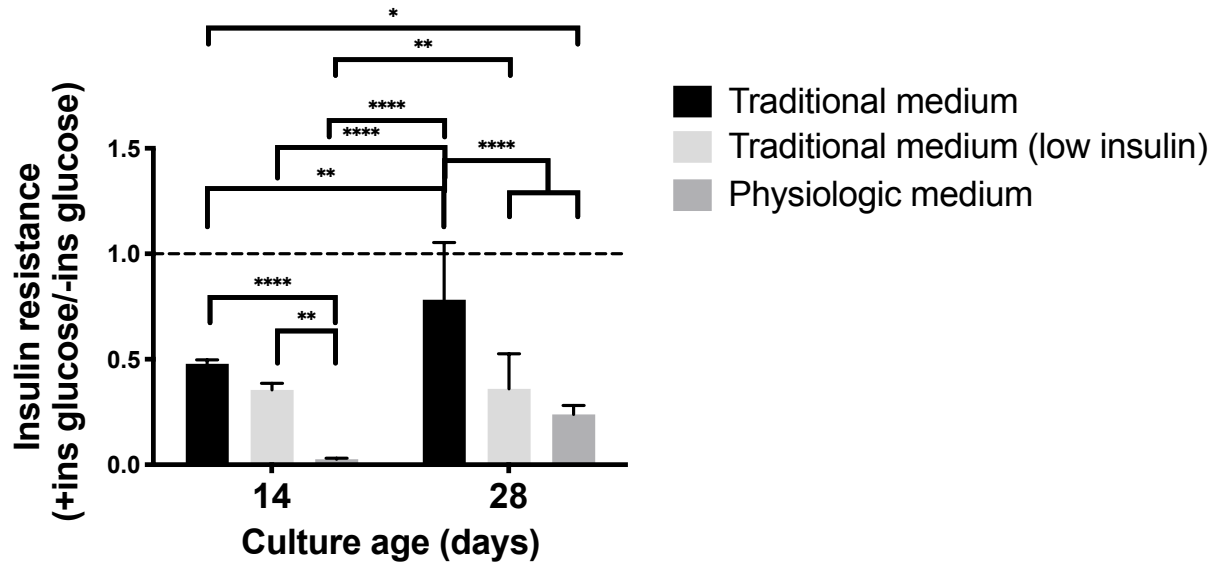
**Supplement Figure 1. Effects of physiologic insulin concentration on primary human hepatocyte (PHH) functions in medium supplemented with 10% (v/v) bovine or human serum.** Urea production of PHHs in micropatterned co-cultures (MPCC) with 3T3-J2 fibroblasts cultured in either (a) bovine or (b) human serum over time in physiologic insulin (500 pM, circles) or high insulin (1µM, squares). Normalized CYP3A4 activity of hepatocytes in MPCCs cultured in either (c) bovine or (d) human serum over time in physiologic insulin or high insulin. Error bars represent SD. \* represents  $p < 0.05$ .



**Supplemental Figure 2. Optimization of serum concentration in cell culture medium.** MPCCs were cultured in medium with different concentrations of serum for 2 weeks. Albumin production in cultures with (a) bovine and (b) human serum at different concentrations. Urea synthesis in cultures with (c) bovine and (d) human serum at different concentrations. Error bars represent SD. \* $p < 0.05$  and \*\* $p < 0.01$ .



**Supplemental Figure 3. CYP1A2 and CYP2C9 activities in MPCCs cultured in the traditional or physiologic medium.** MPCCs were cultured in traditional or physiologic medium for 3 weeks and assessed for (a) CYP1A2 and (b) CYP2C9 activities. Error bars represent SD. \* $p < 0.05$  and \*\* $p < 0.01$ .



**Supplemental Figure 4. Insulin resistance over time with varying insulin concentration and serum type.** MPCCs were cultured in the traditional medium (containing bovine serum) with the normal high insulin concentration (1  $\mu$ M) or physiologic insulin concentration (500 pM), or physiologic medium (containing human serum and physiologic insulin concentration) and assessed for insulin resistance as described in Figure 4 of the main manuscript. This figure shows the same data depicted in Figure 4 for comparison. Error bars represent SD. \* $p$ <0.05, \*\* $p$ <0.01, \*\*\*\* $p$ <0.0001.