

## Figure S1: Filtering applied to select 1,120 multiplex families for analysis.

A series of exclusion criteria were applied to all 2,278 families enrolled in the AGRE collection to select a set of multiple-incidence nuclear families for evaluation of recurrence risk. The number of families passing each filter are shown in green, and the number of families filtered out at each step are shown on the right in pink.



## Figure S2: Patterns of ASD risk to children born after the multiplex criterion is met in families with at least two subsequent births.

After a family has 2 affected children, ASD recurrence risk is less for later-born males, particularly those in MO (male-only) families. Line graphs show the proportion of affected children born first and second after a second affected child in those families with at least two children born after their second affected child by A) sex, B) family type as FC (female-containing) or MO, and C) by sex within each family type. Line plots corresponding to each group are indicated by colors (blue for males, red for females) and solid (FC, female-containing families) and dashed (MO, male-only families) lines. The number of families evaluated in each group, the relative risk (RR) to the child born first versus second after the second affected child, and the p-value from a two-sided Fisher's exact test are shown in the tables below each panel. Whiskers note the 95% confidence intervals around each recurrence rate estimate.