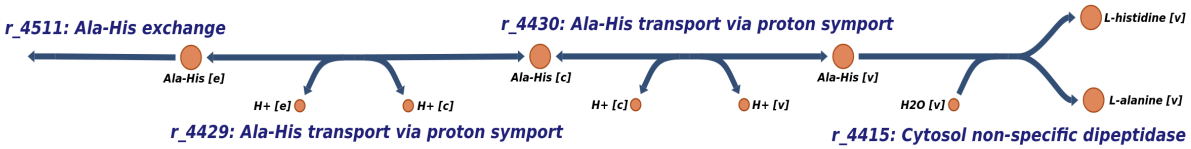
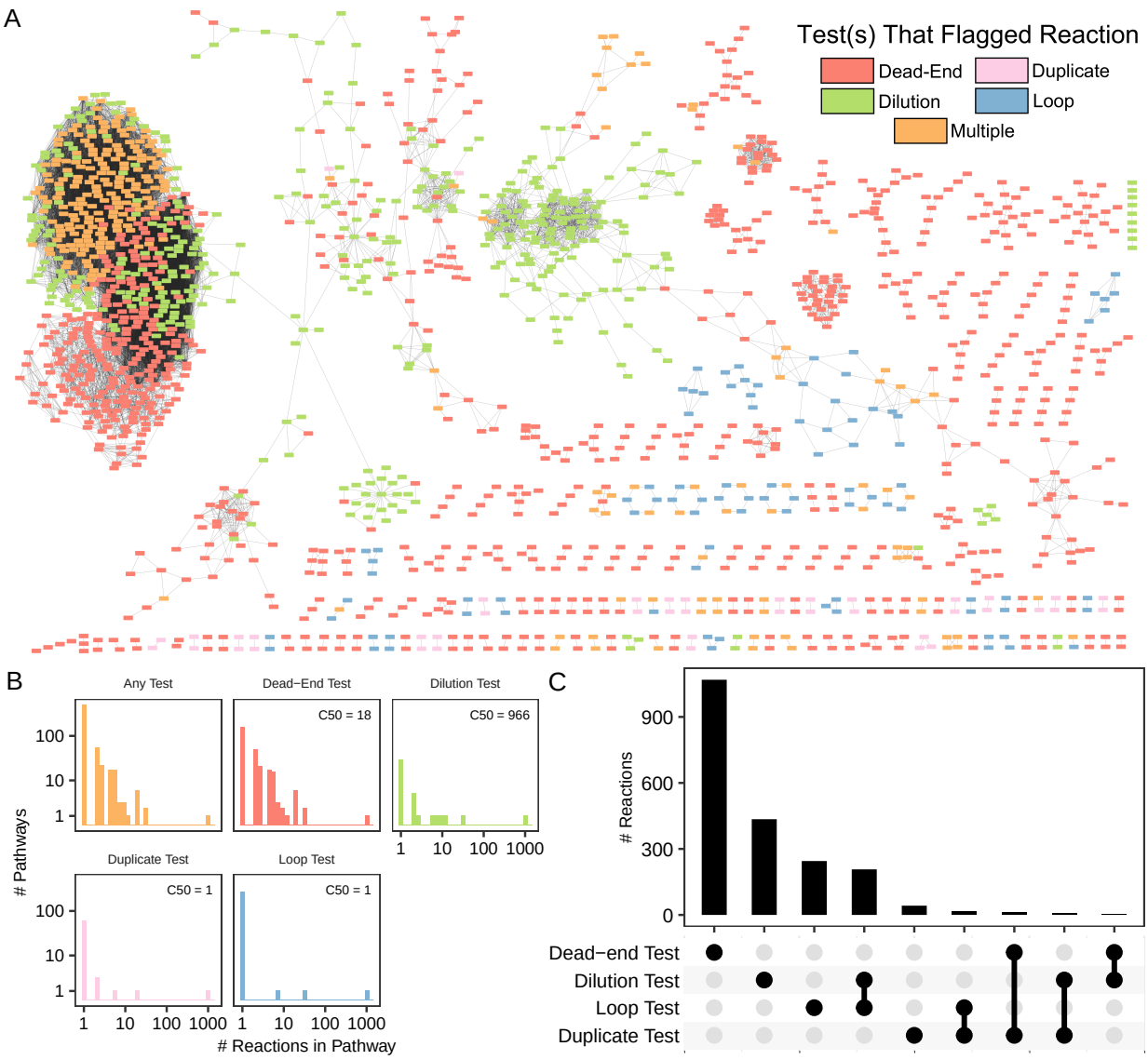
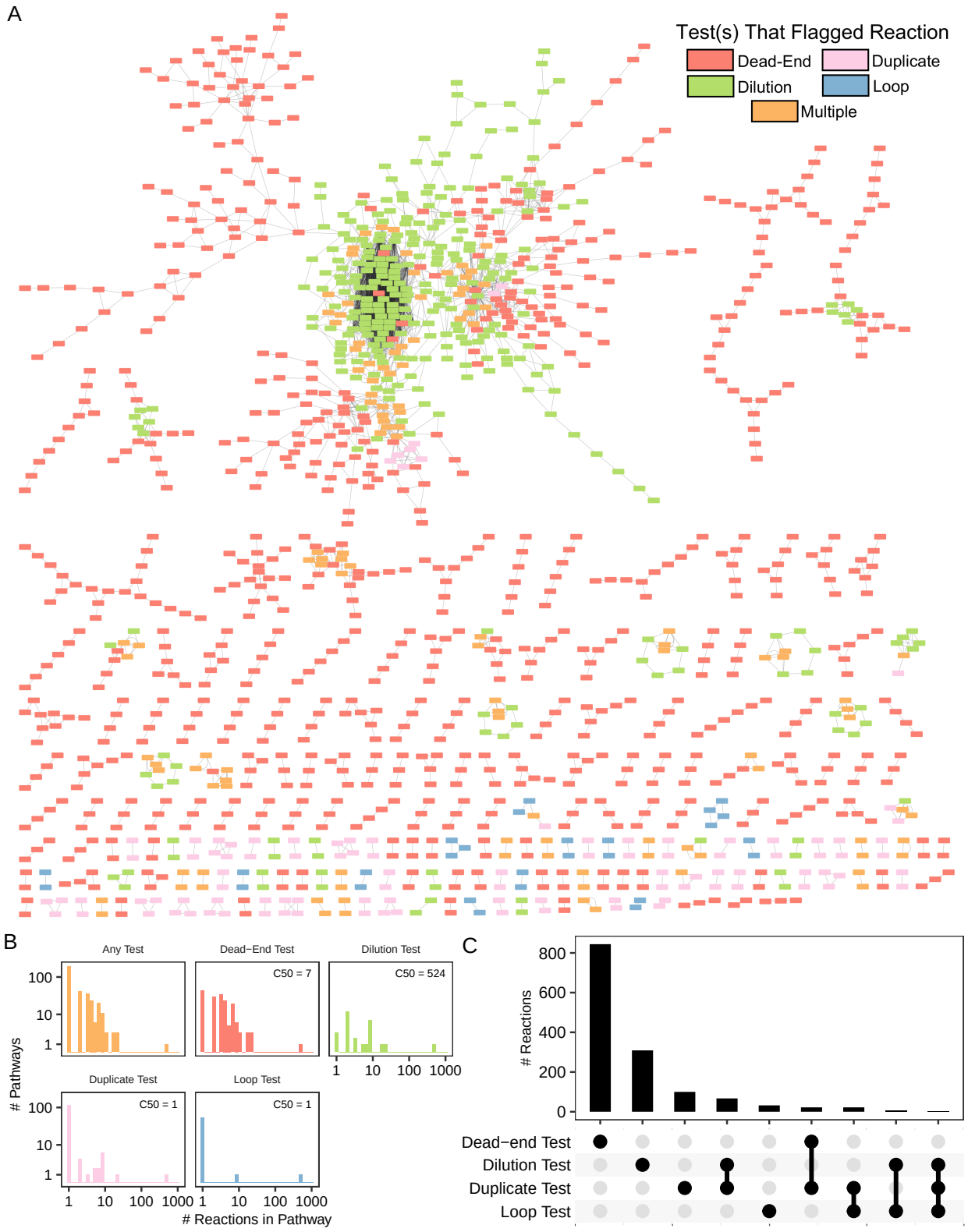


Supplementary Figure 1. Escher map of a pathway of reactions flagged by the dead-end test in version 9.0.0 of yeast-GEM.



Supplementary Figure 2. Overview of reactions in version 9.0.0 of yeast-GEM flagged by one or more tests in MACAW. (A) Each node represents a single reaction; see Methods for explanation of how reactions were connected. The color of each node indicates which test(s) the reaction was flagged by. (B) Distributions of numbers of reactions in each connected component ("pathway") shown in a. for all pathways or only pathways containing at least one reaction flagged by the specified test. (C) UpSET plot showing number of reactions flagged by each observed combination of tests in MACAW.

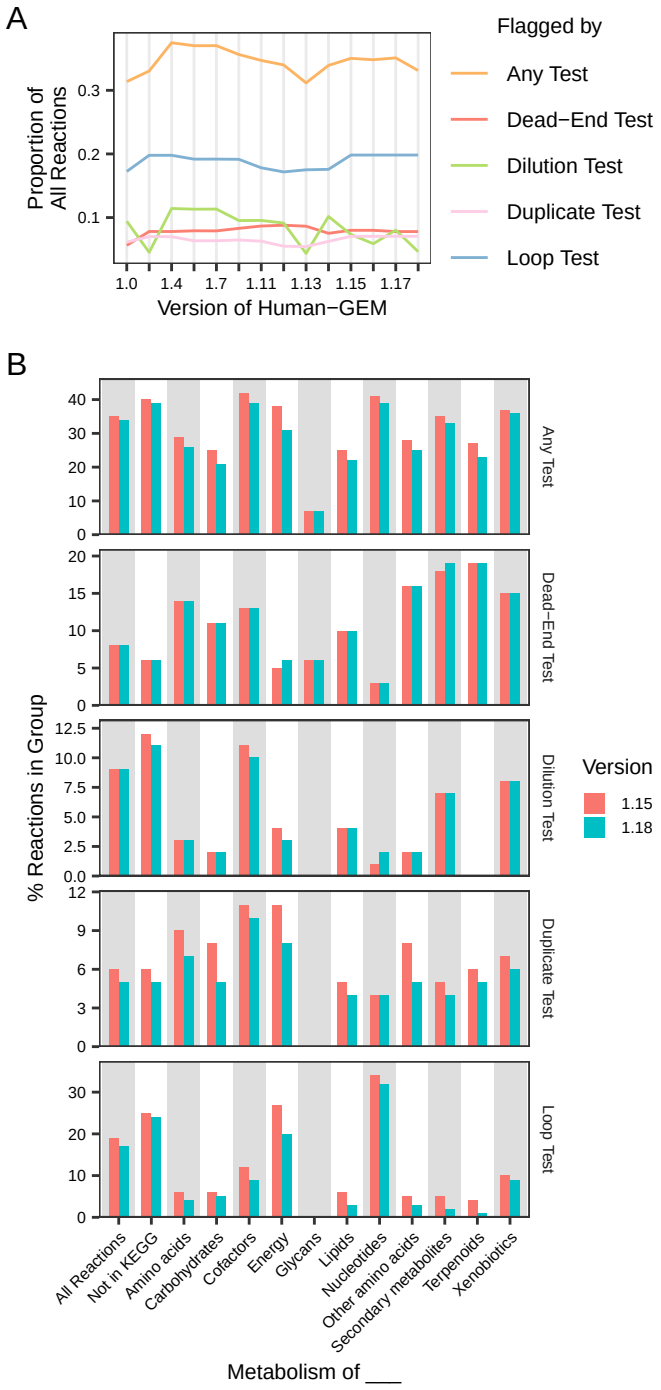


Supplementary Figure 3. Overview of reactions in iML1515 flagged by one or more tests in MACAW. (A) Each node represents a single reaction; see Methods for explanation of how reactions were connected. The color of each node indicates which test(s) the reaction was flagged by. (B) Distributions of numbers of reactions in each connected component ("pathway") shown in a. for all pathways or only

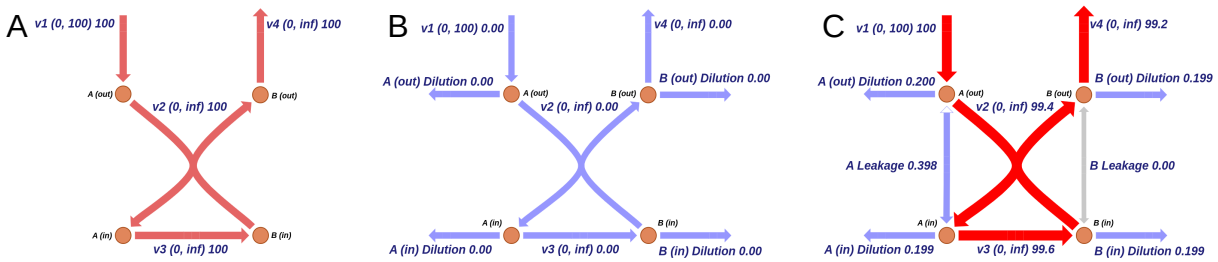
pathways containing at least one reaction flagged by the specified test. (C) UpSET plot showing number of reactions flagged by each observed combination of tests in MACAW.

```
## # A tibble: 14 x 8
##    model_version all_rxns flagged `dead-ends` `dilution-blocked` duplicates
##    <fct>            <int>   <int>       <int>              <int>      <int>
##  1 1.14             13024    4821        1029               1476        827
##  2 1.16             13085    4541        1134               1249        822
##  3 1.15             13073    4658        1085               1246        847
##  4 1.12             13070    4898        1020               1493        914
##  5 1.17             12969    4408        1140               1184        713
##  6 1.13             13026    4821        1031               1474        827
##  7 1.3              14770    5009        1111               1502        923
##  8 1.0              15185    4763         855               1431        930
##  9 1.7              13082    4592        1020               1049        923
## 10 1.11             13069    4317        1021                593        916
## 11 1.18             12995    4051        1123                567        706
## 12 1.9              13081    4331        1020                607        924
## 13 1.4              13096    4587        1047                961        923
## 14 1.5              13096    4559        1047                771        923
## # i 2 more variables: loops <dbl>, redoxes <dbl>
```

```
## # A tibble: 70 x 3
##    model_version test             prop
##    <chr>         <chr>           <dbl>
##  1 1.0           Any Test       0.314
##  2 1.0           Dead-End Test  0.0563
##  3 1.0           Dilution Test  0.0942
##  4 1.0           Duplicate Test 0.0612
##  5 1.0           Loop Test      0.172
##  6 1.3           Any Test       0.339
##  7 1.3           Dead-End Test  0.0752
##  8 1.3           Dilution Test  0.102
##  9 1.3           Duplicate Test 0.0625
## 10 1.3           Loop Test      0.176
## # i 60 more rows
```



Supplementary Figure 4. Test Results Between Different Versions of Human-GEM. (A) Proportions of reactions flagged by tests across all versions of Human-GEM. (B) Proportions of reactions flagged by tests in two versions of Human-GEM. Individual reactions may be associated with more than one group of KEGG functional orthologs. Names of KEGG functional ortholog groups have been abbreviated.



Supplementary Figure 5. "Leakage" reactions prevent the dilution test from flagging unproblematic antiport reactions. (A) Toy model with two metabolites that can exist in two different compartments: "out" and "in", and move between them via the antiport reaction v2. Numbers in parentheses next to reaction labels are the minimum and maximum allowed fluxes through that reaction. Numbers following reaction bounds are the optimal fluxes through each reaction when maximizing flux through v4. (B) Same as (A) except one dilution reaction has been added for each metabolite whose flux is constrained to be exactly equal to 0.1% of the sum of the absolute values of all other fluxes that involve that metabolite. (C) Same as in (B) except one leakage reaction has been added connecting each pair of metabolites that exist in two separate compartments. Fluxes through leakage reactions are constrained to be between -1 and 1.